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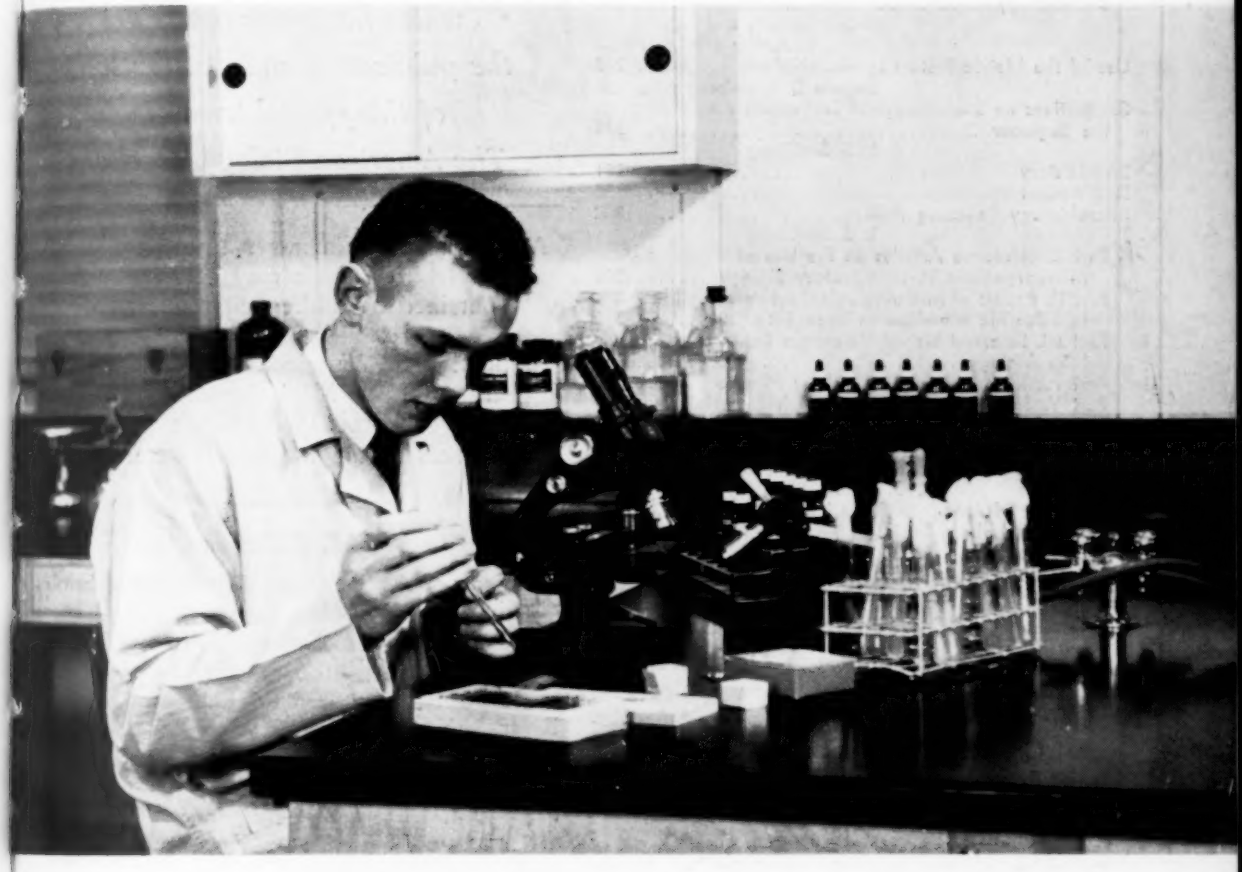

Special Issue

MICROBIOLOGY IN INTRODUCTORY BIOLOGY

Sponsored by

Committee on Education
Society of American Bacteriologists

TABLE OF CONTENTS

From the Society of American Bacteriologists 323
L. S. McClung
Bacteriology Career Information 323
From the National Association of Biology Teachers ... 324
Paul Klinge
NABT Officers 324
NABT Associate Members 324
Experiments with Bacteria in High School Biology 325
Sister M. Paulinus, O. P.
Committee on Education, Society of American Bacteriologists 332
Do It Yourself Microbiology Kit 333
Clarence T. Lange
Local Branches of the Society of American Bacteriologists 336
Bacteriology Inoculating Devices Useful in the Biology Laboratory 337
David L. Fagle and L. S. McClung
Simple Procedure for Making Pipettes for Bacteriology 338
L. S. McClung and Helen R. Arthur
Use of the Metric System in Microbiology 340
Eugene D. Weinberg
Suggestions on Bacteriological Techniques for the Beginner 343
L. S. McClung
SAB History 351
SAB Annual Meetings 359
Microbiology Teaching Aids 352
L. S. McClung
Part I. Reference Articles on the Use of Microorganisms in Introductory Biology 353
Part II. Papers of Microbiological Interest Published in Scientific American in Recent Years 356
Part III. Selected List of Reference Books in Microbiology 360
Part IV. Introductory Bacteriology Films 370
Part V. Supply Houses from Which Bacteriology Supplies May Be Obtained 373
Part VI. Suggestions for Experiments in Bacteriology for Introductory Biology Classes and Student Projects 376
The Society of American Bacteriologists 382
Sustaining Members, Society of American Bacteriologists 383
National Association of Biology Teachers 384

Cover Picture

A student is shown in a Department of Bacteriology laboratory, Indiana University, making slide preparations. Courtesy of Audio-Visual Center, Indiana University.

THE AMERICAN BIOLOGY TEACHER

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of this special issue of
The American Biology Teacher

Additional complimentary single copies of this issue may be obtained by addressing request to Executive Secretary Society of American Bacteriologists 19875 Mack Avenue Detroit 36, Michigan Teachers should use school letterhead.

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In addition to the above the Council of the Society is composed of one Councilor from each of the 31 Local Branches and four Councilors-at-Large.

From the Society of American Bacteriologists

The Society of American Bacteriologists welcomes this opportunity to join with the National Association of Biology Teachers in the publication of this issue of *The American Biology Teacher* and extends appreciation to the Difco Laboratories for the generous grant which permitted the Society to subsidize this special issue. In 1958 the Council of the Society authorized the reactivation of its Committee on Education, and among the charges to the Committee was the development of teaching aids in microbiology for distribution to teachers of biology. This issue reflects the activities of the Committee.

The Society long ago expressed an interest in the introduction of the study of microorganisms in introductory biology. The eleventh President of the Society, V. A. Moore, suggested (Science *33*, 15-16, 1911) that "the courses of biology of our common schools introduce instruction on the existence and varieties of bacteria, and something of the action of a few of the more important economic species. This would afford a little fundamental knowledge to help the masses to better understand the course of many natural phenomena." Even before this, Frost and Hastings had published a series of articles entitled "Bacteriology for High Schools" (Journal of Applied Microscopy and Laboratory Methods, *6*, 2205-2208, 2270-2273, 2383-2385, 2426-2428, 2522-2527, 1903). In retrospect, this seems even more remarkable when it is remembered that the first college courses in bacteriology in this country were offered only in the early 1890's —scarcely two decades after the pioneer experiments of Pasteur and Koch which established this science on a firm basis.

The Society also expresses its interest in the stimulation of interest in science in young students by awarding certificates of commendation to those high school students, and their sponsor teachers, who win awards for exhibits on microbiology at local, regional, and state science fairs and for outstanding papers at programs of Junior Academies of Science. For these certificates, the teacher or principal should forward appropriate details to the Executive Secretary of the Society or the Chairman of the Committee on Education. At the 1960 National Science Fair-International in Indianapolis, a new series of awards, to be known as the Society of American Bacteriologists Awards, will be inaugurated. First and second place awards, consisting of checks for $125 and $75, respectively, will be made for the best exhibits in bacteriology, and in addition, the winners and their schools will receive engraved plaques. For more mature research, each year the Eli Lilly Award of $1,000 and medallion is presented to a bacteriologist under the age of 35 for exceptionally meritorious research.

Membership in the Society is open to teachers of biology who have interest in the objectives of the Society. Applications (see back page of this issue) should be sent to the Executive Secretary who will contact bacteriologists in the area for the necessary sponsoring signatures.

The Committee on Education will welcome comments and suggestions on the material of this issue. These and questions relating to bacteriological problems in introductory biology should be sent to the undersigned.

L. S. McClung, *Chairman*
Committee on Education
Society of American Bacteriologists

Department of Bacteriology
Indiana University
Bloomington, Indiana

Bacteriology Career Information

The brochure *A Career in Bacteriology* may be obtained on request to Executive Secretary, Society of American Bacteriologists, 19875 Mack Avenue, Detroit 36, Michigan. Single copies may be obtained free of cost. Larger quantities are available at 10 cents per copy. A 15 minute film of the same title is also available; an abstract of this is given on page 371.

Requests should be directed to the Executive Sectretary for copies (50 cents each) of a list of colleges and universities which offer programs leading to degrees in bacteriology or microbiology.

From the National Association of Biology Teachers

The National Association of Biology Teachers welcomes the opportunity to join with the Society of American Bacteriologists in preparing and distributing this special issue of *The American Biology Teacher* for the membership of NABT and other interested persons. Especially do we wish to thank one of our loyal advertisers, Difco Laboratories, for its generous support which made this additional issue possible.

This undertaking is another evidence of the cooperative effort being made by professional biologists and teachers in improving the quality of biology instruction in this country. Teachers may feel confident that they have loyal and cooperative friends in the professional biologists.

Especial thanks are due the chairman of the Committee on Education of the SAB, Professor L. S. McClung, Indiana University. His hard work made this issue move from a dream into a reality. He has a long record of keen interest in the problems facing the secondary school biology teacher.

More information concerning the NABT and its work is carried on the back page of this issue, but this extra issue of the ABT represents one of the services which NABT has for its membership. We cordially invite other interested professional organizations to use this medium in bringing to the teacher appropriate information to improve biology instruction.

Again, our thanks to SAB and the Difco Laboratories.

PAUL KLINGE, *Editor*

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Experience with Bacteria in High School Biology

SISTER M. PAULINUS, O. P.,
St. Mary's Convent, Cheyenne, Wyoming

Bacteria are of extreme importance to man. Extensive studies are constantly being carried on in research laboratories to determine the microbiological activities in man's environment. At the present time, bacteriology is one of the most fertile fields of experimental biology. Yet high school biology teachers may not realize the possibilities that the field offers for them. Often the use of bacteria in high school biology is limited to the demonstration of the smallest forms of visible life, or at most to their potential role as pathogens, or their utility in the foods and textile industries. Doubtless the reason has been that experiments in the field are traditionally thought to require elaborate apparatus, expensive microscopes, and advanced techniques. Quite the opposite is true.

The traditional course in biology needs and is being given revision. In many instances, however, high school biology consists only of the study of anatomy, morphology, and classification. This results simply in an accumulation of facts without that stimulation of creative thinking which is really the goal of education, and for which better science teaching at the elementary level is preparing our students.

The less explored side of biology, animal and plant physiology, offers a new and exciting vista. It is in this area that bacteria as experimental organisms can be widely utilized. The ease with which specimens may be obtained and maintained argues for a greater use of bacteriology than is the common practice at the present time.

C. L. Strong in an article in *Scientific American* compares the culture of microorganisms to farming in miniature. This is indeed true, given the soil, the seed, and the right environment, the fruit is plentiful. The joy of bacteriology is that with a minimum of effort one can easily control all three factors.

The first consideration, then shall be the "soil," or the medium. Several recent articles for beginning bacteriologists have advocated the use of dehydrated media. This may be obtained from biological supply houses, such as the Baltimore Biological Laboratory and Difco Laboratories. The advantages are apparent. One needs only to rehydrate, tube, and sterilize to have a ready source.

One of the disadvantages which no one has pointed out, aside from the cost, comes from the necessity of ordering from supply houses which are not ordinarily dealt with, or buying in large amounts when little is needed. The necessity of buying several different types of media also poses a problem. If, however, several different types of media are not used, the advantage of utilizing bacteria to demonstrate physiological processes is lost, a very real loss.

The medium commonly used for growing bacteria is nutrient broth or its solid equivalent, nutrient agar. Many other types are just as usable, supply all the growth factors necessary, and at times are much more easily obtained. Several of these media will be described. Most are empirical; the only recommendation that can be offered for them is that they are successful. It might very well be that they would work equally well in lesser amounts, or better in larger amounts. Experiments to test quantities have not been run, because many of the experiences which the students have with bacteria do not require standardization of media as will be seen later with the discussion of these experiences.

The following media we have used with success:

Dried Beans. About a half cup of any kind of beans may be boiled in a liter of water and filtered, and the soup or broth used. Water can be added to the soup so that the end product will equal a liter. This broth may be tubed and sterilized. If a solid medium is required, add a 2% solution of agar (20 grams of agar in a liter of medium). Some do use a 1.5% solution with success; we find a 2% solution more satisfactory.

Soil Extract. Plant factors, as we all know, are

Homemade spectrophotometer made with photoelectric cell mounted in light tight box and connected to galvanometer. Anthony Perella and William Finnerty. L. to R.

found in abundance in rich soil. A cup of soil is leached in a liter of water. After filtering, the water can be used as a liquid medium, or it can be solidified, if desired, with 20 g. of agar. This makes a very clear medium for some studies.

Vigoro. One tablespoon of Vigoro in a quart of water is sufficient. This medium is selective; some types of bacteria do not grow well on this. For this reason it is not suitable for counts, but it demonstrates a synthetic medium, and it is reproducible for continued studies with some species.

Malted Milk. Two tablespoons full of plain malted milk powder (without chocolate), as purchased on the market, can be used either filtered or unfiltered, as a broth or solid, depending on the need.

Carrots or Potatoes. Carrots or potatoes, diced, boiled and filtered, used either as broth or as a solid medium, are easily procured and can be used extensively for counts, isolating, or as a stock culture medium.

Nutrient Broth. As has been stated before, nutrient broth has been used traditionally. It is desirable to use this medium in many experiments because the results are known. Meat extract, such as can be purchased at a grocery store or a meat market for use in gravies or soups, makes a very satisfactory nutrient broth. The fact that this is already salted does not seem to interfere. Since salt is only bacteriostatic above two percent, (a much higher concentration is necessary before it is bactericidal), and the concentration in most of these preparations is lower, there seems to be no need to worry. The salt concentration is sometimes listed on the label. If there is a choice, we prefer one with a lower concentration. One half teaspoon of the extract in a liter of water is usable.

Bouillon Cubes. Bouillon cubes have been suggested at various times and are satisfactory in a limited way. Ordinarily one cube in a liter of water is suggested. It is our experience that this often will not sustain growth. Two or three cubes in this amount of water is needed; in this amount growth is abundant. Here again some attention must be paid to the salt content. It is not amiss to say that in trace amounts some salt is helpful. In the bean, carrot, and potato infusion we ordinarily include 0.1 gram of salt, but this is not necessary. In most synthetic media, a small amount of sodium chloride is commonly included.

Milk. For showing acid fermentation, milk, with a small amount of litmus or some other indicator with a pH range of about 7 can be used. The milk, with the indicator in it, is tubed and sterilized. Each tube is inoculated with one type of bacteria. Sometimes, when several different species are used in as many tubes, a whole spectrum, ranging from blue through the neutral lavender to pink can be seen after a day's incubation. It will be found easier to dissolve the litmus in water first, and then add the solution to the milk. These are merely suggestions. The ingenuity of the teacher will find many other things which are easily obtained in a

Agar is stored in medicine bottles and melted in tin cans. Terence O'Donnell.

given situation. To ignore these is to cut off a fertile source of supply.

Moreover, since most of these media supply the fundamental food factors they can often be used for other purposes as well. We grow the gametophyte generation of ferns and mosses from spores on petri dishes of soil agar medium. Since they can all be made up and sterilized simultaneously, it saves much time in an overcrowded schedule. Once students have made up media, with a minimum of supervision, they can and do take care of it almost entirely.

From the foregoing, it can be seen that in many cases it is advantageous to order a pound of agar at the drug store or from supply houses and to use it for any or all of these media. This is one of the advantages over buying dehydrated or ready-made media: it can be easily obtained. One pound of agar usually costs from five to six dollars and lasts us, with extensive use, from two to three years.

A point to be remembered in making a solid medium is that after the medium is made up and the agar added, the whole should be boiled gently until all of the agar is dissolved. If the agar is not completely dissolved before sterilizing, difficulties arise. Moreover, if the medium is not clear after the agar is boiled, it should be filtered before tubing, lest the particles be mistaken for colonies and erroneous counts result. Ordinarily No. 2 filter paper or paper towel is suitable for filtering. We use a plug of glass wool in a funnel, since it permits more rapid filtering.

Gelatin may also be used to solidify the medium. In this case, care must be taken that the bacterium does not use gelatin in its metabolism, or the temperature of the incubator must be below 28° C to prevent liquefaction. This is easily noticed if primary isolation is made from a petri dish of gelatin since the gelatin liquefies around those colonies of bacteria which use it. It is easy, however, to pick colonies from the solid portions of the gelatin. Still it is worth stressing that many experiments can be made in liquid media.

Colony counts made on photographic print box. Patricia Scherer, Judy Sargent. L. to R.

Medicine bottles are generally used instead of petri dishes. Brigitte Carls, Judy Sargent.

The medium, either broth or agar, can be tubed in ordinary test tubes, and cotton plugs used. These are easily handled and have the advantage of having just about enough medium for one 100 mm. petri dish. Ordinarily, about twelve-fifteen milliliters of medium is put in an eight inch test tube. It saves considerable time in tubing if this amount is measured into one tube and the others in the rack filled to the same level, the medium being poured through a funnel to which a rubber tube with a pinch-cock is attached.

If the medium is liquid, we use test tubes because they are inoculated and read without transfer. However, small medicine bottles with plastic screw tops, such as may be purchased at the drug store for four cents each, are in many cases more satisfactory for solid media. In filling the bottles care should be taken that they are not filled more than half-full because of the expansion which takes place during sterilization. One, two, six, and eight ounce bottles are used, the larger sizes being used to store media, or when several petri dishes are to be filled at the same time.

More and more we use small medicine bottles in place of petri dishes, and they have

Home canning pressure cooker on electric plate used for sterilization. Fachon Schwope, Judy Sargent.

been found just as suitable. They have, in addition, the extra advantages in high school use, of being much less expensive, not nearly so easily broken, and much more easily stored. Except for initial isolation of bacteria or molds from the air, we use them almost exclusively; we even make counts in them.

About seven to ten milliliters of agar medium is poured into the two ounce bottles, or 20 ml. in the six to eight ounce bottles, and, after sterilization, allow to solidify either in a slant or flat, on the curved side of the bottle, opposite the graduation. The slants are used to demonstrate growth characteristics such as surface contour, color or antibiotic reactions; those which are flat, such things as colony characteristics.

Since most counts in high school work are merely comparative anyway these can be quite satisfactorily done in bottles. As previously indicated, the agar is allowed to solidify on one side of the bottle. For this the sample (dilution of milk, etc.) is added to the liquefied (but cooled to about 40° C) agar before the bottle is placed on its side. Bottles of the same size, with the same amount of agar are used and the number of colonies between the lines which mark the graduations is counted. Any colony which is partly under the line is disregarded.

Sterilization is comparatively easy in the pressure cooker. All sizes have been used here, but we find the type used for home canning to be most suitable. Ordinarily it is heated over the electric plate, which though slower, is safer and can be easily watched during a laboratory or class period. When this is done, the students are alerted to the danger involved and one or two are appointed to glance at the pressure gauge occasionally. The advantage of an electric plate, of course, is that there is no open flame to worry about, and if connected through a rheostat, the pressure can be kept at fifteen pounds almost indefinitely. It is generally accepted that complete sterilization is achieved in fifteen to twenty minutes at fifteen pounds of pressure.

Sometimes we have made up a large amount of media at the same time and taken it to a nearby hospital to be sterilized in the autoclave.

We seldom use distilled water for media, unless an experiment demands it. If a great deal of distilled water is needed, for instance for individual projects, the hospital or a local bottling works is a good source of supply. Should it be obtained from a bottling works, it is important to make sure that the water is truly distilled, not just demineralized. Distilled water is never required, although many formulae will list this item, for making nutrient broth or agar or when the medium contains complex plant or animal products.

When placing test tubes to be sterilized we usually cut the top out of a number 2½ tin can, punch a few holes in the bottom to insure a flow of steam around the test tubes, place either paper towels or glass wool on the bottom to minimize breakage, and lower the tubes into the can. These cans of test tubes are readily handled for storage. While filling, if the cans are tilted on the side against a book,

Synthetic formulae easily made up by students with minimum of help. Fachon Schwope, foreground.

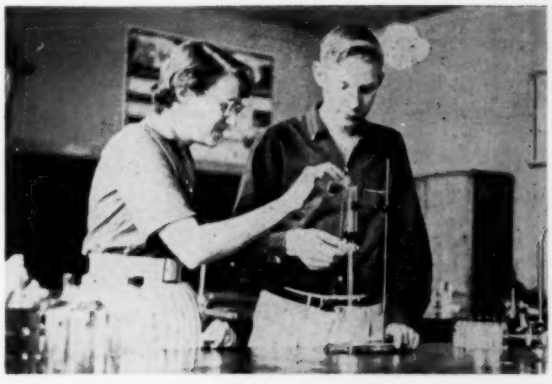
the test tubes are more easily slipped into the can. Care must be taken, however, not to tilt the tubes in such a way that the cotton plugs are allowed to become wet.

Medicine bottles can also be placed in tin cans or wire baskets. In sterilizing the bottles, the screw tops are left fairly loose to provide for expansion and are tightened after the bottles have been removed from the autoclave.

Perhaps the greatest difficulty for most people in working with bacteria comes from fear of disease. With ordinary care this fear is unnecessary. Few pathogenic bacteria grow easily at room temperature, and since all of the experiences described later are geared to growth without an incubator, this fear is largely unfounded. Nevertheless, each student should be alerted to the danger and warned to wash his hands with soap and warm water after working with the organisms, and in no case to put his hands near his face or into his mouth during the work. This is no more than common sanitary laboratory procedure, however.

After use, the liquid media may be sterilized in the pressure cooker before disposal, or emptied directly down the drain and flushed with plenty of water, or the bacteria may be killed with formaldehyde, of which there is usually a superabundance from the shipment of other specimens. Solid media we almost always place in the pressure cooker, since this is the quickest way to melt it, and empty it down the drain with plenty of water. Thus both melting and sterilization is achieved. The utensils are always washed with hot soapy water.

Petri dishes wrapped in paper towels and placed in pressure cooker. Ronald Sullivan, Fachon Schwope, Brigitte Carls.



Separatory funnel used in isolation of enzyme from culture. Judy Sargent, John Maraldo.

Comparing the study to farming in miniature, we have just completed preparing the "soil." The "seed" cultures may be obtained from the agricultural department of the state university, or from the American Type Culture Collection in Washington 7, D. C., 2112 M Street, N.W., or some few may be purchased from some of the biological supply houses. However, for the beginner, the disadvantages of sending away for and maintaining cultures are again encountered.

Since few pathogenic bacteria remain alive long outside the body, we find it satisfactory to expose a petri dish of agar medium to the air for about ten minutes in an unoccupied room. After incubation in a dark, warm place, many colonies will have grown. The dishes are allowed to incubate from 48 to 72 hours, since color does not ordinarily show up at first, and the colored colonies are more interesting to the students. Colonies may also be interesting because of their contour, shape, or luster.

Colonies which are interesting and isolated are picked off with a wire needle into a medicine bottle slant. Incubation again for a period of about 48 hours is allowed, or until good growth takes place. This time is not critical. Usually the fluctuation of room temperature does not permit good growth in twenty-four hours.

If petri dishes are not available, small beakers or glass dishes may be used with aluminum foil for a cover.

After the colonies have been permitted to grow in bottles of nutrient agar for three or four days, they can be stored in a refrigerator for two to three months without transfer. If

Ordinary glass tubing serves as pipette. Accuracy of calibration sufficient for most high school work. Brigitte Carls, John Maraldo, Judy Sargent.

the tops are screwed tight there is neither danger of contamination to the culture nor to the other things stored in the refrigerator, nor is there probability of their drying out in this length of time. A few drops of glycerin put into the medium before sterilization will also prevent drying during storage. When agar in test tubes is going to be kept for several days or a week, it is equally helpful to place a few drops of glycerin in the medium to prevent drying.

Transfer of bacteria can be made with loops of wire. Some have advocated using dissecting needles for this. We do not find them handy. Usually the wire is too stiff for convenience. If meat skewers, obtained from a butcher, are softened by allowing them to remain overnight in water, a number 20 or 22 nichrome wire can be forced into them with a pliers. We find a length of two and a half inches usable since it can easily be sterilized without burning the handle.

The studies that are possible with bacteria are endless; the life processes are readily demonstrated. In fact, it is by means of these studies that I teach the meaning of digestion, nutrition, metabolism and enzyme relationships, concepts which are seldom clearly understood otherwise.

To show the effect of enzymes on sugars, a one to five percent solution of the sugar in nutrient broth is made. The solution should be tubed and a small test tube or vial inserted upside down into the larger tube. During sterilization the smaller tube will fill with the solution. Inoculated with bacteria and allowed to incubate for twenty-four hours, the broth becomes turbid. With some types of bacteria if the organism uses the sugar gas bubbles will form in the small tube.

When the sugar is utilized, acid is usually formed which can be demonstrated by the use of an indicator, added either before sterilization or after incubation. Litmus, brom thymol blue, or some other indicator having an end point close to 7 can be used. The acid produced is of course an excretory product and elucidates the excretory process, in counter-distinction to egestion of undigested particles, which even more advanced students often find hard to distinguish.

If, as was indicated with the discussion on litmus milk, several tubes are inoculated simultaneously with different species, a comparison can be made and the physiology more easily demonstrated.

Several sugars are readily obtained; sucrose (table sugar), dextrose and lactose (from any drugstore), or fructose from fruit juices. Karo and maple sugar are also simple sugars which can be used. It is of course the utilization of the lactose in the milk which brings about the change in the litmus, although occasionally the litmus itself may be reduced in which case the milk turns white again.

It is possible to obtain enzymes, cell free, by centrifuging the broth culture and decanting the supernatant fluid. This fluid can be added to the sugar solution and after standing, preferably but not necessarily in a warm water bath, tested with an indicator. Observation of pH changes and sometimes gas bubbles may be made during the interim. This is a simple test which can be performed by each student, small four inch test tubes being used, the

Dilutions and counts become routine for some studies. Terence O'Donnell, Brigitte Carls.

warmth of the hand being sufficient to hasten the reaction.

The traditional, and still one of the best, demonstrations of enzymatic reaction is that of hydrolysis of sugar by the supernatant fluid from a centrifuged culture of yeast, using Fehling's solution to show the hydrolysis.

Protein degradation and utilization may be shown by liquefaction of gelatin. It seldom happens that from several soil bacteria obtained from the air in the manner described above, at least one will not show this property of being able to liquefy gelatin.

The existence of the enzyme which catalyzes the breakdown may be demonstrated by making a thin broth of gelatin, (a five cent package of Knox gelatin works beautifully), growing the bacterium in it and centrifuging the culture. The supernatant may then be put on a drop of gelatin, which will usually liquefy within a few minutes. We use either a microscope slide with a well, or a four inch test tube, and each student is provided with a drop of gelatin to observe.

Protein degradation may be shown also by the burnt cork odor of nutrient broth, but since most soil bacteria degrade many proteins, this is not good. Many of the more sensitive students find the odors very distasteful.

Another enzyme which can be readily demonstrated is catalase. Catalase is an enzyme which is found extensively in aerobic bacteria. Chemically it is related to the hemoglobin in the blood, and its function is similarly respiratory, having to do with the transfer of oxygen or the release of oxygen from hydrogen peroxide. To test for it one needs merely to add hydrogen peroxide to a small amount of the supernatant fluid obtained in any of the above demonstrations, or to a loopful of the bacteria. The release of bubbles of oxygen is proof of the presence of catalase. This reaction also may be shown on a microscope slide, but I prefer to use the small test tubes, again letting each student participate. Individual observations can be encouraged on this and many of the experiments because one cubic centimeter of the material is amply sufficient for the test. It is a tremendous help in the study of respiration.

Since the amount of enzyme present is always in direct proportion to the rapidity of the reaction which it catalyzes, we have made

Medicine bottles are easily inoculated. John Maraldo, Brigitte Carls, Judy Sargent.

quantitative and comparative tests on catalase production of several bacteria. To do this we place five cubic centimeters of hydrogen peroxide in a eudiometer tube and fill the remainder of the tube with the supernatant from the centrifuged nutrient broth culture. As the hydrogen peroxide mixes, with the inversion of the tube into a small dish of the supernatant, bubbles of oxygen rise to the top of the tube. By measuring the amount of oxygen which rises in a given time, five to twenty minutes, a good comparison can be made.

The breakdown of complex carbohydrates can be demonstrated by testing the utilization of starch. A small amount of soluble starch mixed in cold water and then into boiling water is added to nutrient agar before sterilization. It is plated and bacteria streaked with the tip of a needle across the surface of the agar, making several parallel streaks. After incubation, the agar is flooded with tincture of iodine. If the starch has been utilized, a clear zone remains around the colony while the unused starch gives the typical blue test with iodine. Many of the soil bacteria show this characteristic. In addition, several different kinds of starch are available: potato starch from the vegetable or from laundry starch, wheat starch from flour or wall paper paste, and corn starch.

From the foregoing, it is easy to see many other applications. Some identification of bacteria is easily possible without a microscope. Observation of growth characteristics and observation of characteristics of colonial growth can be made macroscopically.

The bacteriostatic effect of antibiotics is commonly urged and easily carried out.

Physical phenomena as they affect life may be studied; temperature, effects of sunlight, light intensity, or the lack of light, varying wave lengths of light, ultraviolet wave lengths, electricity, electrophoresis, magnetism and a comparison of one to the other, showing the similarity of cause with the similarity of effect.

From all this it might seem that we do nothing else but a year-long study of bacteria. Quite the contrary; they are merely used in addition to other forms, to introduce or to clinch an idea. Because of the unity which exists among all living things, bacteria showing those basic processes by which life itself is sustained can be used in many areas.

Bacteria not only offer a ready source of living organisms, a constant supply of demonstration and experimental material any time during the year, but they require less time, less space, and less care than practically any other animal or plant. A stock culture can be made in a medicine bottle, the top screwed tight, the culture placed in the refrigerator, and a demonstration organism is ready at a moment's notice. With a minimum of instruction students can prepare media which, after it is sterilized, can be kept in screw top bottles almost indefinitely. Bacteria lend themselves to many experiments in which a microscope is unnecessary. Moreover, because of the rapidity with which bacteria multiply, reactions are also rapid, so that a problem can be presented and solved in one period, and demonstrated on an individual basis. In a changeable adolescent world, interest is aroused and satisfied before it can lag. To interest a student is to motivate that student, and a motivated student is one who achieves.

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Sources of Supply

Baltimore Biological Laboratory, 2001 Asquith Street, Baltimore 18, Maryland.

Difco Laboratories, Inc., Detroit 1, Michigan.

American Type Culture Collection, 2112 M Street, N. W., Washington 7, D. C.

Committee on Education Society of American Bacteriologists

1959-1960

Harold J. Blumenthal, Department of Bacteriology, University of Michigan Medical School, Ann Arbor, Michigan.

L. Leon Campbell, Department of Microbiology, School of Medicine, Western Reserve University, Cleveland 6, Ohio.

David Fagle, Marshalltown Senior High School and Junior College, Marshalltown, Iowa.

Milton J. Foter, Sanitary Engineering Center, U. S. Public Health Service, 4676 Columbia Parkway, Cincinnati 26, Ohio.

David Hendlin, Research Division, Merck and Company, Inc., Rahway, New Jersey.

Clarence T. Lange, State Department of Education, Jefferson Building, Jefferson City, Missouri.

*Leland S. McClung, Department of Bacteriology, Indiana University, Bloomington, Indiana.

Quentin Myrvik, Department of Microbiology, University of Virginia, Charlottesville, Virginia.

Raymond W. Sarber, Executive Secretary, Society of American Bacteriologists, 19875 Mack Avenue, Detroit 36, Michigan. (*ex officio*)

I. L. Shechmeister, Department of Microbiology, Southern Illinois University, Carbondale, Illinois.

William G. Walter, Department of Botany and Bacteriology, Montana State College, Bozeman, Montana.

Delaphine G. R. Wyckoff, Department of Botany and Bacteriology, Wellesley College, Wellesey 81, Massachusetts.

*Chairman of Committee.

Do It Yourself Microbiology Kit

CLARENCE T. LANGE
Science Consultant, Missouri State Department of Education, Jefferson City

Can your biology class be called a laboratory course in science? Do you have difficulty finding the time to assemble the required materials to conduct laboratory exercises? The following describes a compact kit which contains most of the items required to support laboratory work for a unit on microbiology. The kit as pictured in Figure 1 measures 25″ by 10″ by 10″. The contents of this kit were assembled for about $100.00. By using substitutes as suggested herein, the cost can be held to less than $50.00.

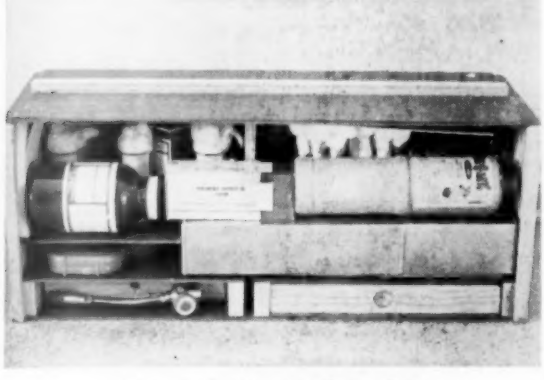


Figure 1. Bacteriology kit.

This kit embodies portability, as evidenced by the fact that the author carries the kit throughout the state of Missouri for use in conducting teaching workshops. The convenience of having most of the materials in one case is evident for small school systems. The portability aspect should have merit where these materials are to be used and moved from classroom to classroom in a large district.

Contents of Kit

The items listed were chosen to minimize breakage since the kit is transported. Local conditions of use may require the inclusion of fewer numbers of each item or smaller quantities of the expendables with the coincident smaller initial cost for assembly of the kit. Figure 2 shows the following items: propane gas burner; culture tubes; 1 ml., 5 ml., and 10 ml. pipettes; pipette box; petri dishes (plastic); 250 ml. flasks; nutrient agar; potato dextrose agar; nutrient broth; antibiotic assay disc set; set of 16 pure cultures; dry yeast packets; American Type Culture Collection catalog; Society of American Bacteriologists bacteriology lists; inoculating loop; forceps; microscopic slides; wax marking pencil; spatula; plugging cotton, and transparent tape.

Figure 2. Contents of bacteriology kit.

Prepared materials in the kit are as follows: 1200 ml. of sterile agar; 25 sterile pipettes (assorted); 24 sterile agar slants; 14 sterile water blanks; and 14 sterile tubes of nutrient broth.

The gas burner was chosen rather than an alcohol burner because it is a better heat source for sterilization and melting agar, also it is relatively unbreakable. The culture tubes with screw caps are expensive. Reasonable substitutes are culture tubes plugged with cotton. Commercial pipettes offer the greatest convenience, but adequate substitutes can be prepared by drawing lengths of glass tubing to a point in a flame, then break open the point. These lengths of glass tubing can be calibrated to deliver 1 ml., 5 ml., and 10 ml. amounts. Presterilized plastic petri dishes offer a great convenience to instructors who are hard pressed for time. 500 sterile plastic petri dishes can be purchased for an amount equal to the cost of three dozen glass petri dishes. Glass dishes pose the problem of breakage and facilities for cleaning and sterilization. In this kit 250 ml. flasks are used for preparation and storage of agar. Prescription bottles of ade-

quate size can be used as substitutes. Small packages of dehydrated media offer a diversity of choice and convenience in preparation. The set of antibiotic treated paper discs can be obtained for less than two dollars per vial of 50 treated discs. Substitutes can be had by dissolving known antibiotic pills or capsules in sterile water, and then soak discs punched from filter paper in this solution. Antibiotic samples are generally available from local druggists or doctors. The American Type Culture Collection, 2112 M Street, N. W., Washington 7, D. C. has special rates for educational institutions for their pure cultures. This kit contains the following pure cultures: *Aerobacter aerogenes*, *Agrobacterium tumefaciens*, *Alcaligenes faecalis*, *Aspergillus niger*, *Bacillus brevis*, *Bacillus megaterium*, *Bacillus stearothermophilus*, *Bacillus subtilis*, *Escherichia coli*, *Penicillium notatum*, *Proteus vulgaris*, *Pseudomonas fluorescens*, *Saccharomyces cerevisiae*, *Salmonella pullorum*, *Sarcina lutea*, and *Staphylococcus albus*. Local state colleges and universities are frequently in a position to provide cultures for interested instructors. I have found that city or state departments of health frequently have some of the more common cultures and will cooperate with local instructors in providing same. This kit includes a set of lists prepared by the Society of American Bacteriologists which include material regarding experiments, references, films, and equipment relating to teaching bacteriology. These are published in this issue.

Prepared Media and Materials in Kit

Figure 3 illustrates the materials required for media preparation. Since the graduate cylinder and balance are general laboratory equipment, no effort was made to include these in the kit. The pressure cooker for sterilization of media or glassware is an item generally available in a home economics department or in the home of the instructor or some interested student. The completed kit contains four flasks of sterile nutrient agar and two flasks of sterile potato dextrose agar. The nutrient agar is for work with bacteria, and the potato dextrose agar for work with the fungi.

Figure 3. Materials for media preparation.

Figure 4. Glassware preparation

Both glass and plastic petri dishes are shown in Figure 4. If the glass dishes are used they can be wrapped in newspaper and sterilized in an oven at 350° F. for three hours. If the dishes are wrapped 3 or 4 per package before sterilization they will remain sterile until unwrapped for use. Aluminum foil wrapped pipettes are also sterilized in the oven. Nutrient broth tubes containing 10 ml. each, agar tubes containing 10 ml. each, and 9 ml. water blanks in tubes are prepared. These tubes are sterilized in the pressure cooker with the flasked agar 15 minutes at 15 lbs. pressure. After sterilization, the agar tubes are placed on a slanting surface to cool. This procedure provides the agar slants required in isolation techniques.

Simple Experiments Using Kit

Figure 5 shows how the materials in the kit can be used in making studies of the number and kinds of microorganisms present in our environment. Tests can be made using soil samples or samples of foods. One of the drawers in the kit is made to accommodate standing sterile water blanks used in making dilutions as indicated. One ml. samples are drawn from the desired dilution tubes and

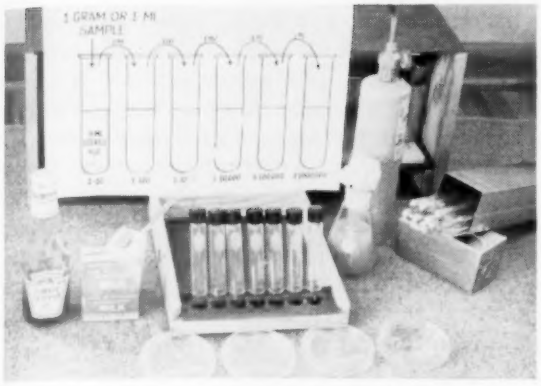


Figure 5. Dilution technique for study of numbers of microorganisms in environment.

placed in marked petri dishes. Cooled molten agar is added, the plates gently swirled to facilitate mixing, and then are set to incubate. Following appropriate incubation, counts of the bacterial colonies in a plate multiplied by the dilution factor gives an indication of the number of bacteria in the original sample.

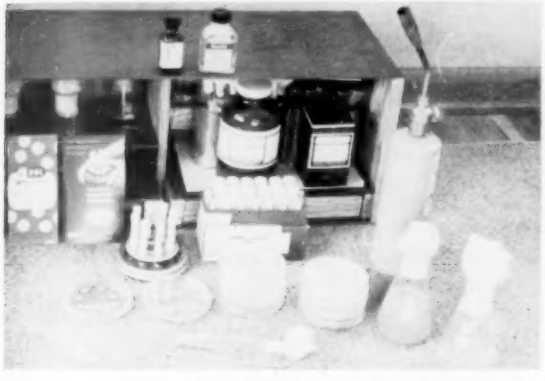


Figure 6. Test of antibiotics or household antiseptics against known organisms.

Figure 6 shows the setup for making antibiotic assay tests, as well as tests with paper discs soaked in household antiseptics or antibiotic solutions made from pills or capsules. A pure culture loop full of bacteria is mixed with a flask of molten but cooled agar. Plates are poured, and the various paper discs, antibiotic assay, or class-prepared discs, are placed on the solidified agar surface. After incubation, clear areas or zones of inhibition around a disc indicate antibacterial activity regarding the material on the disc.

Figure 7 is the setup of an experiment for studying the metabolism of yeast. The left hand graduate cylinder was set up with one

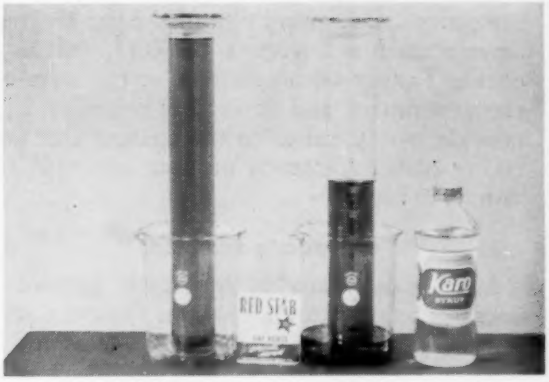


Figure 7. Metabolism of a living cell using yeast.

package of dry yeast and warm water. The filled cylinder was inverted as in the picture. The right hand graduate cylinder was set up similarly with 50 ml. syrup, a package of yeast, and warm water. The evolution of gas has pushed the water to the level indicated.

Figure 8. Parts required for homemade incubator.

Record at 10 minute intervals the total gas evolved as the yeast cells utilize the food for

Figure 9. Incubator in use with plates and culture tubes.

life processes. A graph plot of the gas evolved against time will give a typical population curve. Tasting both solutions at the start of the experiment and at regular intervals will provide some clues as to the changes that are taking place. A record of taste observations should be kept.

Homemade Incubator

Microbiology studies frequently require a constant temperature incubator. Figures 8 and 9 show a reasonable incubator well within the finances of most schools. The author used a camp ice box, although any suitable wooden box would do. The heater element is a heat tape of the type used by house trailer dwellers to keep water pipes from freezing. This tape is wound around a corner slotted cardboard box. The thermostat is of the egg incubator variety available at most farm supply stores. When assembled, as shown in Figure 9, and connected with an extension cord, an incubator with an even and uniform source of heat is obtained.

Local Branches of the Society of American Bacteriologists

Branches are organized on a geographical basis, depending on the concentration of bacteriologists, and indicated in the list below. The Branches hold at least two meetings each year and often the programs contain items of interest to other biologists. Associate membership in the Branch is usually available to those who are not active members of the National Society. Teachers are encouraged to investigate the program of the Branch in their area.

Allegheny: E. S. Lindstrom, Department of Bacteriology, Pennsylvania State University, University Park, Pa.

Northern California-Hawaii: Carl Lamanna, Naval Biological Laboratory, Naval Supply Center, Oakland 4, Calif.

Southern California: A. F. Rasmussen, Jr., Department of Infectious Diseases, UCLA Medical School, Los Angeles 24, Calif.

Colorado-New Mexico-Wyoming: R. DeBoer, Bacteriology Laboratory, St. Luke's Hospital, 601 East 19th Ave., Denver 3, Colorado.

Connecticut Valley: R. C. Cleverdon, Department of Bacteriology, University of Connecticut, Storrs, Conn.

South Florida: M. M. Streitfeld, National Children's Cardiac Hospital, Miami 34, Fla.

Illinois: H. B. Harding, Northwestern Medical School, Chicago, Ill.

Indiana: Harold Garner, Department of Bacteriology, Purdue University, West Lafayette, Ind.

Intermountain: R. D. Sagers, Department of Bacteriology, Brigham Young University, Provo, Utah.

Kentucky-Tennessee: R. W. Beck, Department of Bacteriology, University of Tennessee, Knoxville, Tenn.

Maryland: F. Ray, Technical Evaluation Division, Fort Detrick, Md.

Michigan: Marvis Richardson, Route 1, Box 469, East Lansing, Mich.

Eastern Missouri: J. T. Barrett, Department of Microbiology, School of Medicine, Columbia, Mo.

Missouri Valley: L. W. Hedgecock, Veterans' Administration Hospital, 4801 Linwood, Kansas City, Mo.

New Jersey: F. C. Kull, Research Department, Ciba Pharmaceutical Products, Inc., Summit, N. J.

Central New York: L. E. Bowers, Department of Bacteriology and Botany, Syracuse University, Syracuse 10, N. Y.

Eastern New York: W. D. Bellamy, 1159 Phoenix Avenue, Schenectady, N. Y.

New York City: J. R. Lucker, 83 Burton Ave., Woodmere, Long Island, N. Y.

North Carolina: Mary Poston, Duke Hospital, Durham, N. C.

North Central: J. C. Ayres, Food Processing Laboratory, Iowa State College, Ames, Iowa.

Northeast: Alice Marston, 80 East Concord St., Boston 18, Mass.

Northwest: Jane Nishio, Rocky Mountain Laboratory, Hamilton, Mont.

Ohio: B. A. Steinberg, William S. Merrell Co., Cincinnati 15, Ohio.

Eastern Pennsylvania: Elizabeth Fowler, Temple University, Philadelphia 22, Pa.

Puerto Rico: Francisco J. Landron, Professional Building, Santurce, Puerto Rico.

Rio de Janeiro: M. A. Fuks, Fac. National de Farmacia, Univ. do Brasil, Rio de Janeiro.

South Central: J. D. Schneidau, Jr., Department of Microbiology, Tulane University Medical School, New Orleans, La.

Southeastern: Margaret Green, Department of Bacteriology, University of Alabama, University, Alabama.

Texas: E. O. Bennett, Department of Biology, University of Houston, Houston 4, Texas.

Virginia: W. A. Volk, Department of Microbiology, School of Medicine, University of Virginia, Charlottesville, Va.

Washington, D. C.: Elizabeth Oswald, Division of Antibiotics, Food and Drug Administration, Washington 25, D. C.

Bacteriology Inoculating Devices Useful in the Biology Laboratory

DAVID L. FAGLE
Marshalltown High School and Department of Biology, Marshaltown Junior College, Marshalltown, Iowa

and

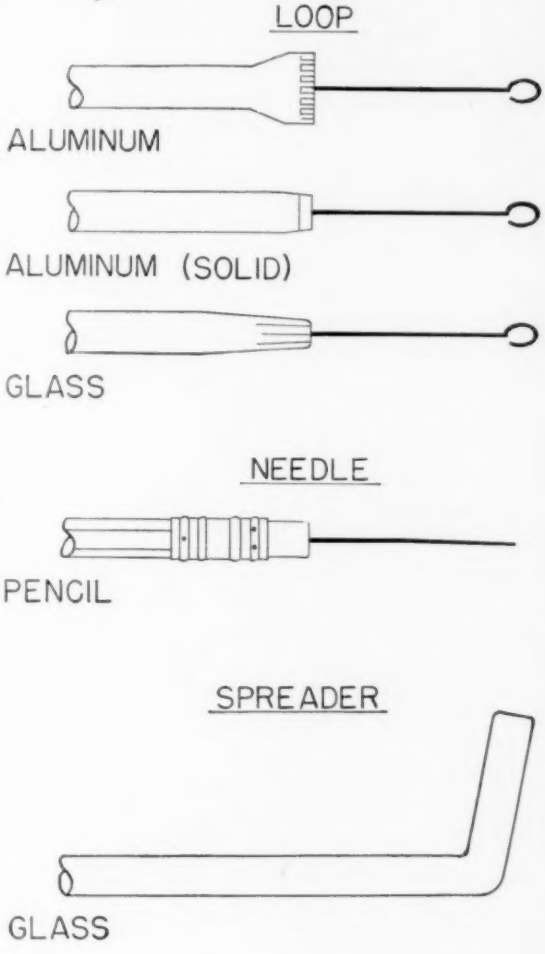
L. S. McCLUNG
Department of Bacteriology
Indiana University, Bloomington

One of the problems of teaching bacteriology at the high school or freshman college level is the expense of many bacteriology items. With a little ingenuity teachers can improvise much of the material needed for a unit or course in elementary bacteriology. Since many bacteriology experiments require plated inoculated media, some information concerning inoculating devices may be helpful to teachers.

There are four simple methods of inoculating a culture medium: the loop method, the "hockey stick" or spreader method, the swab method, and the pipette method.

Wire inoculating needles, or transfer loops, are used for making streak plates, nutrient broth, or agar stab cultures depending upon the type of culture desired. These may be purchased from any of the major biological supply houses, but needles and loops that are just as efficient may be improvised. A three to four inch piece of nichrome wire (B and S 24 gauge) may be secured in the end of a piece of thin (one-quarter inch or less in diameter) aluminum tubing by flattening the end of the tube around the wire to hold it secure. Solid aluminum rod, first seen by the junior author at the University of Wisconsin in 1932, may be substituted for the tubing. For this, with a hand drill and with #52 or #56 twist drill bit, drill a ¼" to ⅜" long hole in one end, insert wire, and then flatten end. The wire and aluminum tubing or rod may be purchased from most industrial equipment and supply companies.

A second method of inoculating loop construction is to use a glass tube and insert the nichrome wire into the bore of the tube; then with heat close the end of the glass tube around the wire. Solid glass rod may also be used by heating end of rod until it is soft, inserting wire, and taking care to anneal rod (deposit carbon on heated portion by holding in yellow gas flame as an aid in preventing cracking).



As suggested by Fagle (1956) a very simple but efficient inoculating needle may be made by fixing, with a pair of pliers, a piece of nichrome wire (and you may substitute a regular sewing needle for the wire) in the eraser of a regular pencil. The "handle" of this improvised needle cannot be sterilized in the flame as is desirable, but the device is useful if other needles are not available.

At times it is desirable to have cultures cover the entire plate of medium. In this case a "hockey stick" or spreader may be used. This is made by taking an eight-inch glass rod, ¼" diameter or less, heavy aluminum wire, or coat hanger wire, and bending a right or obtuse angle about one and one-half inches from the end. Before using this spreader, it must be sterilized either in hot air, by autoclaving, or by flaming after dipping in alcohol. Similar devices may be fashioned from pipe cleaners; these cannot be flamed but may be sterilized either by dry or moist heat. After a drop of liquid inoculum is placed in the center, the spreader is used to make a smear plate much as a paint brush would be used to paint the surface of the culture medium.

In other instances when the wire inoculating loop is not suitable, the swab method of inoculation may be used. Two types of swabs are available for this technique. Sterile "Q-tips" may be obtained from most drugstores, but a small piece of cotton twisted on the end of a toothpick, or swab stick, may be used after sterilization by dry heat in the oven. The sterile swab is dipped into a vial or tube of sterile tap water and then charged by rubbing across a surface, such as a dirty dish or other utensil, or colony, on agar. With liquid cultures, the sterile swab may be dipped in the culture to obtain the inoculum, and the plate is then inoculated by the smear technique.

Often liquid-to-liquid transfers are needed. Although the needle or loop may be used, sometimes it is convenient or necessary to use a sterile pipette for inoculation. In the absence of factory made pipettes which may be used for this purpose, a "Pasteur pipette" may be made by drawing out soft glass tubing, 6 to 8 mm. outside diameter, to a capillary tip. This type of pipette, already drawn out, has become available recently through the laboratory supply houses, but pieces of scrap glass may provide an inexpensive substitute. The open or large end should be plugged loosely with cotton, and then the pipettes, several wrapped together in paper, are sterilized in the oven.

References

Fagle, David L., *Handbook of Bacteriology for High School Teachers;* Master's Thesis, Iowa State Teachers College, Cedar Falls, Iowa, 1956.

Simple Procedure for Making Pipettes for Bacteriology

L. S. McCLUNG and HELEN R. ARTHUR
Department of Bacteriology, Indiana University, Bloomington

Many laboratories are unable to afford a sufficient supply of precision, factory calibrated pipettes to permit extensive use in introductory laboratory exercises. This is particularly true for bacteriology since for most purposes the used pipette must be sterilized, washed, and resterilized before being used again. It is not difficult to make and calibrate pipettes for which the accuracy is more than adequate for such purposes. The procedure to be described was adapted from a technique first used by the senior author in 1932 in the Department of Bacteriology at the University of Wisconsin. The many requests for demonstration of the procedure prompts this report.

For the calibration apparatus the following will be required:

(1) 2 unpainted boards, approximately 24" x 6" x 1", one of which is nailed in a secure fashion to stand upright in the center of the other as shown in Figure 1.

(2) 3 pieces of intravenous (thin wall) rubber tubing; bore size depends on the type of calibration tube but should be not less than ¼ inch.

(3) 1 pinch clamp.

(4) 1 clamp or eyelet.

(5) Small supply of mercury.

(6) 1 calibration tube. This may be a 2 ml. factory made pipette or burette but must be capable of accurate measurement from a 0 point to 1.0 ml. and then to 1.1 ml. The 2.2 ml. pipette (Corning Glass, Pyrex #7056) is ideal using the 1 ml. graduations as the 0 point.

The above items are assembled or used as in Figure 1 and as indicated below. Attach a 4 inch length of rubber tubing to the top of the calibration tube to prevent overflow of mercury. Attach a 12 to 16 inch piece of rubber tubing to the bottom of the tube to serve as a mercury reservoir. To this, attach a 4 inch length of glass tubing, 8-10 mm. diameter, and to the glass tube, another 6 inch length of rubber tubing. A pinch clamp is used on the last short length of tubing to prevent loss of mercury when the pipette calibrator is not in use. The purpose of the glass tubing is to provide a rigid section to which you attach an eyelet or clamp. It is important that the eyelet be secured tightly and that it has an opening of some type that will hook over a screw or nail. If this eyelet should vary in position, you will get a false reading when calibrating the pipette. Secure the calibrated tube to the wooden support with wire. It is good to cushion the wire with small pieces of rubber tubing to prevent breakage of the calibration tube, and it is most important that the tube does not shift or slip after being placed into position. You are now ready to place the wood screws to determine the calibration points. Hold the eyelet parallel or slightly above the 1.1 calibration and add mercury to the top of the calibration tube until the column rises to the 1.1 ml. position. The pinch clamp should be open or removed at this time. Place a wood screw into the board (Position A) so the eyelet will fasten over it and maintain the column at the 1.1 ml. position. Repeat the procedure for the 1 ml. (Position B) and O positions (Position C). Check the position of the mercury column each day before using the calibrator. Add or remove part of the mercury depending on the barometric pressure.

The pipettes are made as follows: Cut 6 or 7 mm. (O.D.) soft glass tubing into 2 ft. lengths. Heat the tubing, rotating to secure even heating, at center point over Bunsen

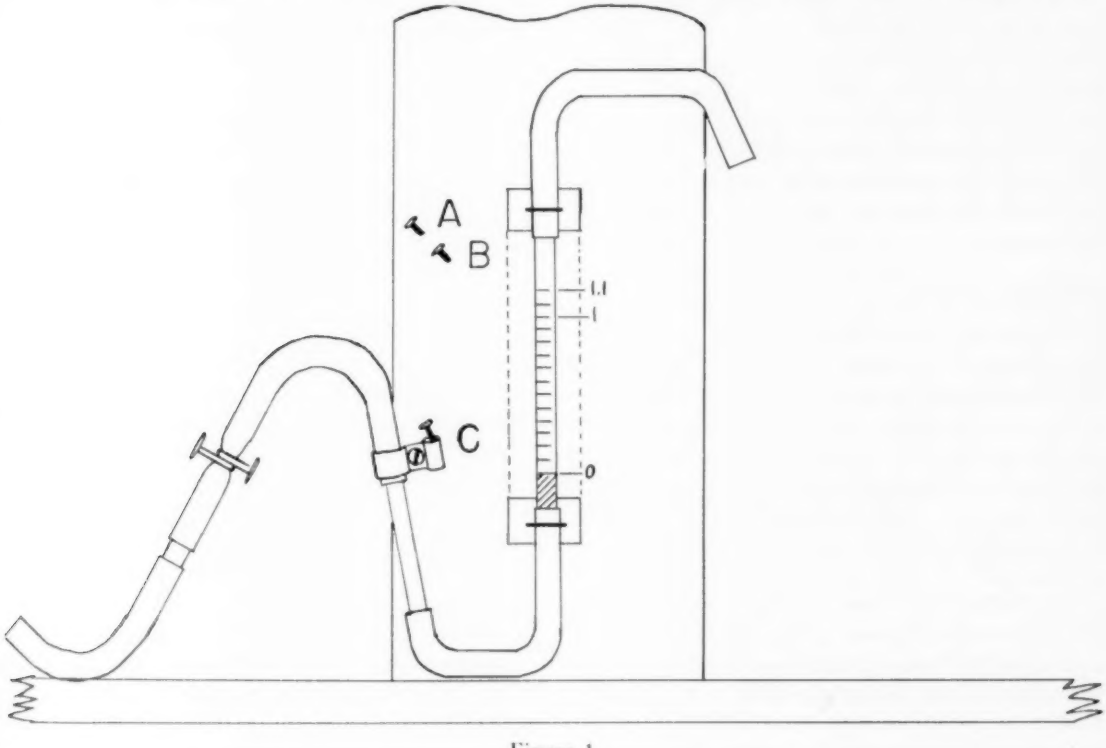


Figure 1

burner and draw approximately 1 inch. Place aside to cool. When cool, etch center of drawn portion and break. Fire polish both ends of pipette—both tip and mouth ends should be clean-cut. To calibrate the pipette, place a beaker or container of water to the left of the calibrator. It is best if the container of water is at a level 4 to 5 inches below the base of the calibrator. Place the eyelet at the Position C. Remove the pinch clamp. Attach the rubber tubing to the mouth end of the pipette and place the tip of the pipette in the water. Raise the eyelet to the Position B and mark the level of the water with a wax (or china marking) pencil the point of which has been sharpened to permit a fine mark on the pipette. Then move the eyelet to Position A and again mark the pipette. Return the eyelet to Position C and the pipette should be completely cleared of water. At intervals check that the meniscus of mercury column is at the proper graduation mark with the eyelet in Positions A, B or C.

To etch the calibration on the pipette, we use a "Handee Grinder," Central Scientific Co., Model #88585, ($32.95) with a thin-edged abrasive wheel. Mount the grinder on a ring stand at approximately a 45° angle and hold the pipette so lightly against the abrasive wheel that the wheel turns the pipette around. A stirring motor or any small type motor may be adapted for this purpose.

Use of the Metric System in Microbiology

EUGENE D. WEINBERG

Department of Bacteriology, Indiana University, Bloomington

Measurements of biological materials in the laboratory usually are accomplished with units of the metric system. Students raised in countries that have adopted the metric system for commercial as well as scientific activities are, of course, familiar with this system from early childhood. However, the majority of students enrolled in schools in the United States have had little or no contact with the metric system, and the following brief discussion is presented for their assistance.

Units of Length.

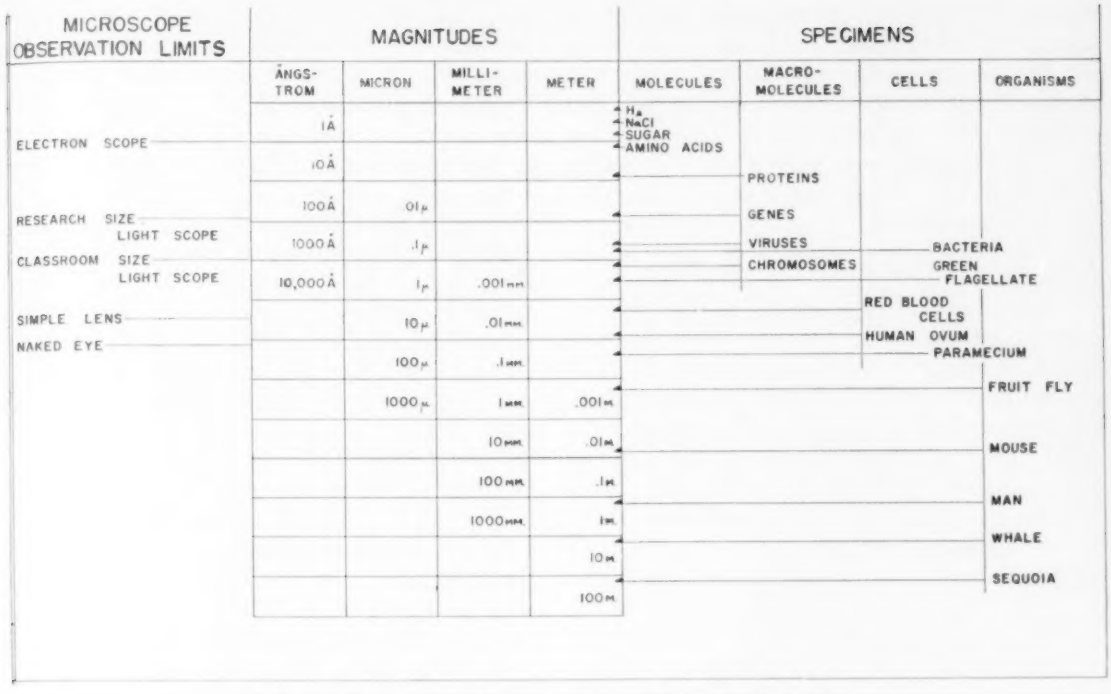
The basic unit of length is the meter. The dimension of the meter is one ten-millionth of the distance from the equator to the poles along any meridian and was selected because of its convenient length. It is approximately three inches longer than a yard. One meter is divided into 100 centimeters (cm.) or into 1000 millimeters (mm.) One millimeter is divided into 1000 microns (μ) or into 1,000,000 millimicrons ($m\mu$). One millimicron is equivalent to ten Ångstrom units (Å).

Objects whose linear dimensions are less than 200 microns usually must be magnified so that we can observe their image. A good compound microscope has a power of magnification up to 1000X; therefore, we can use this instrument to study objects whose linear dimensions are greater than 0.2 micron or 200 millimicrons. The diameter of a human red blood corpuscle is 7 to 8 microns, and a typical spherical bacterium is 1 micron. Small wonder that the development of such sciences as histology and microbiology were greatly accelerated by the invention of the compound microscope!

To visualize some of the numerous objects of biological interest whose linear dimensions are less than 200 millimicrons, an electron microscope can be employed. This instrument can magnify objects approximately 100 times over the compound microscope. Thus, photomicrographs of objects whose linear dimensions exceed 2 millimicrons can be obtained. The presently known viruses range between 10 and 300 millimicrons in length and width, and, therefore, an electron microscope is a valuable and usually necessary instrument in virus research.

Units of Volume.

The basic unit of volume is the liter (L.). One liter contains the quantity of 1000 cubic centimeters (cc.) of distilled water at its greatest density; *i. e.*, at a temperature of 4°



Adapted from "Life," Simpson, Pittendrigh, and Tiffany.

Centigrade at sea level. A cube that contains one liter measures approximately 4 x 4 x 4 inches and occupies a volume of slightly less than one quart.

One liter is divided into 1000 milliliters (ml.). In biological studies, one milliliter (ml.) is considered to be equivalent to one cubic centimeter (cc.). One milliliter can be divided into smaller portions depending upon the precision of available pipettes. Some pipettes for "routine" use are constructed to deliver accurately as small a volume as 0.01 milliliter, and for special research there are available pipettes which deliver even smaller volumes. An average drop from a standard medicine dropper is roughly equivalent to 0.05 milliliter.

Units of Weight.

The basic unit of weight is the gram (gm.). One gram is the weight of one cubic centimeter of distilled water at its greatest density. A liter of such water weighs 1000 grams or one kilogram which is equivalent to about 2.2 pounds. One pound is equal to 454 grams.

One gram is divided into 1000 milligrams (mgm.) and one milligram is divided into 1000 micrograms (μg.) or gammas. One microgram is divided into 1000 millimicrograms (mμg.). Such materials as vitamins, hormones, antibiotics, and minerals usually affect biological systems even when present in less than single gram quantities. The adult human daily requirement of thiamine (vitamin B_1) is estimated to be one milligram, and an effective therapeutic dose of penicillin can be as low as one microgram per milliliter of blood. The quantities required in microbiological studies are, of course, much smaller; one microgram of thiamine, and as few as ten millimicrograms of penicillin, can, respectively, stimulate or poison sensitive bacteria in one milliliter of culture medium.

Even with the most precise analytical balances, it is not possible to weigh accurately quantities of materials of less than ten milligrams. Rather, a larger amount is weighed, dissolved in a known quantity of a solvent such as water, and then diluted in the culture medium to obtain the desired concentration. For example, 100 milligrams of thiamin can be weighed and dissolved in 100 milliliters of water; the concentration is thus one milligram per milliliter (1.0 mgm./ml.). One

tenth of a milliliter of this solution can be added to 9.9 milliliters of culture medium; the final concentration of thiamine in the medium is therefore ten micrograms per milliliter (10 μg./ml.). Since one milliliter of water weighs one million micrograms, a concentration of 10 μg./ml. is sometimes called 10 parts per million.

Concepts of Molarity and Normality.

Concentrations of solutions of biologically active compounds are frequently measured in terms of molarity (M.). The molar concentration of a solution is defined as the number of gram-molecular-weights dissolved in sufficient water to produce one liter of solution. For example, a one molar solution of NaCl can be prepared by adding sufficient water to 58.5 grams of the salt to obtain a final volume of one liter. Or, 5.85 grams of NaCl plus water to obtain 100 milliliters would likewise be termed a one molar (1 M.) solution. Each milliliter of a one molar solution of NaCl contains 58.5 milligrams of the salt. Likewise, each milliliter of a one-thousandth molar (0.001 M or 1×10^{-3} M.) solution of NaCl contains 58.5 micrograms of the salt. When comparisons are to be made of the activities of substances of widely differing molecular weights, concentrations should be expressed in molarity rather than as milligrams or micrograms per milliliter. If molarities are not employed, the compounds of lower molecular weight will give the illusion of being more active than those of higher molecular weight. A very complete list of atomic and molecular weights is contained in the "Handbook of Chemistry," Handbook Publishers, Inc., Sandusky, Ohio.

The use of molarity as a unit of concentration does not provide a basis for direct comparison of all solutions. For example, ten milliliters of a one molar solution of sodium hydroxide (NaOH) can neutralize ten milliliters of a one molar solution of hydrochloric acid (HCl), but only five milliliters of a one molar solution of sulfuric acid (H_2SO_4). The reason for this is that sulfuric acid supplies twice as many hydrogen ions as does hydrochloric acid. In order to compensate for this difference in activity, solutions of alkali and acids are often described in terms of normality rather than molarity. A one normal (1 N.) solution is that portion of a one molar solution that furnishes or reacts with one gram-atomic-weight per liter of hydrogen ions. Thus, a one molar solution of sodium hydroxide or of hydrochloric acid (which would contain 40 grams or 36.5 grams per liter, respectively) would likewise be considered a one normal solution. On the other hand, a one molar solution of sulfuric acid (98 grams per liter) is a two normal (2 N.) solution.

Concept of pH.

In studies of biological materials, a knowledge of the amount of acidity or alkalinity of the solutions being used is essential. The numerical scale used to express degrees of acidity or alkalinity is called the pH scale. A pH of 7.0 represents a neutral solution; numbers below 7.0 refer to logarithmically increasing degrees of acidity, numbers above 7.0 to logarithmically increasing degrees of alkalinity. The pH of vinegar, for example, is 2.25, of water is 7.0, and of milk of magnesia is 10.5. The entire scale ranges from zero to 14, but the majority of living cells prefer a pH close to neutrality.

The pH of a solution can be determined easily by electrometric or colorimetric procedures. For electrometric measurements, an instrument referred to as a pH meter can be used. In many laboratories this instrument is not available so that colorimetric measurements are usually employed. For such measurements, one or more indicator dyes are required. These dyes can be dissolved in the solution or contained in filter paper which is dipped into the solution. Three pH indicators that are quite useful in microbiological studies are brom cresol purple, brom thymol blue, and phenol red. The first turns from yellow to purple between the pH range of 5.2 to 6.8, the second from yellow to blue between 6.0 and 7.6, and the third from yellow to red between 6.8 and 8.4.

Suggestions on Bacteriological Techniques For the Beginner

L. S. McCLUNG
Department of Bacteriology, Indiana University, Bloomington

For those who have not had training in bacteriology, casual visits to the laboratories in which extensive work is being done might indicate that the techniques necessary to the various procedures are difficult to master. Such is not the case, and in this article a few pointers will be given to guide the beginner. A good rule to remember is that "Practice Makes Perfect." Do not expect to be proficient the first time.

Many valuable suggestions, some of which will be repeated or expanded in this article, will be found in A. C. Lonert's, *Turtox Bacteriology Booklet, An Introduction to Bacteriological Methods*, published in 1946, and sold, $1.00, by General Biological Supply House, 8200 South Hoyne Avenue, Chicago 20, Illinois. For additional material, the following books are recommended:

Conn, H. J. (Ed.) *Manual of Microbiological Methods*, McGraw-Hill Book Company, Inc., New York, 1957.

Skerman, V. B. D. *A Guide to the Identification of the Genera of Bacteria*, Williams and Wilkins Company, Baltimore 2, Md., 1959.

Tanner, F. W. *Practical Bacteriology: An Introduction to Bacteriological Technic*, 2nd ed., John Wiley and Sons, Inc., New York, 1933.

These books, which contain valuable suggestions on formulae of media, preparation of staining solutions, staining techniques, etc., assume that the user has had some training in bacteriology. With the suggestions given below, they should, however, be very valuable to the beginner.

Although microscopic examination of bacteria provides much valuable information, it should be remembered that many experiments may be done involving cultivation of bacteria which do not involve the use of a microscope. If microscopic examinations are attempted, the microscope should be equipped with an oil immersion objective. If the school does not possess this, microscopes so equipped may often be borrowed from a local physician, hospital, or industrial laboratory.

Many of the techniques discussed in this article are illustrated in the films, *Bacteria: Laboratory Study* and *Elementary Techniques in Bacteriology*. Abstracts of these films are included in the microbiology Teaching Aid Lists on page 370 in this issue.

Use of Microscopic Preparations

For detailed examination of microorganisms in the living condition, microscopic preparations of two types may be made. For the *wet mount*, all that will be needed will be clean slides and cover slips. These may be used repeatedly if they are thoroughly washed after use with Bon Ami or a similar cleaning agent. Slides should be sufficiently clean that a drop of water will spread in a thin film instead of remaining as a drop, and frequently new slides are covered with a film of oil which must be removed before use. To prepare a wet mount, use pipette tip or inoculation loop to place a large drop of the liquid culture or infusion, or make dilute suspension of colony material in drop of tap water, and carefully lower cover slip in such a manner that no air bubbles are trapped underneath it. If the drop is too small for a coverslip, air bubbles are almost inevitable, but the amount of liquid should not be so great that the cover slip appears to float. In this event absorb excess liquid with pieces of blotter.

A second type of wet preparation, a *hanging drop suspension*, employs a concavity slide, usually of same thickness as ordinary slide, and coverslip. Place a ring of petrolatum (vaseline) around the edge of the concavity and add coverslip on which drop has been placed in such a manner that drop hangs suspended in concavity. The wet mount will ordinarily remain satisfactory for examination for 10-15 minutes; the hanging drop, protected from evaporation, will last for a longer

period. To examine either of these preparations, use the low or high power objective of the microscope. The usual failure to obtain an image results from too much illumination; if microscope is provided with a diaphragm this should be partly closed. The beginner might practice with 48 hour old hay or pepper infusions until success is attained before attempting to study the more dilute bacterial cultures.

For preparation of *stained smears*, use an inoculation loop to spread a thin film of liquid culture in an area about one-half inch in diameter on the clean slide. If colony material from agar culture is to be examined, place a drop of tap water (finger tip is satisfactory for this purpose) on slide and, with the inoculation *needle*, make *dilute* (barely touch colony) suspension. Use a tooth pick or wooden swab stick if the inoculation needle and loop are not available. Allow material smeared on slide to dry in air (2-3 minutes is usually sufficient) and heat-fix to slide by passing slide (smear side up) through bunsen or alcohol lamp flame two or three times. Such a preparation must be stained for best examination.

The books listed above and most introductory textbooks of bacteriology will give directions for a variety of staining procedures, but many substitutions may be made. Gentian or crystal violet is often available at the drug store and is recommended for this purpose. Make a solution of 2 grams of the dye dissolved in 20 ml. of alcohol (iso-propyl or rubbing alcohol is satisfactory) and dilute this with 80 ml. of water. Use 5 ml. of this concentrated solution (which will keep for long periods) and 95 ml. of water to make the final staining solution. To use as a stain, add (usually from dropper bottle) the stain solution directly to smear, and let stand at room temperature for 1-2 minutes. Wash slide with gentle stream of tap water, or use plastic or glass chemistry wash bottle, or "dunk" slide in tumbler of water. Hold slide with blunt tipped forceps to avoid staining fingers.

If supply funds do not permit purchase of dyes for staining procedures, try colored inks as a substitute. The permanent purple inks, in our tests, have been the most satisfactory. For these the time of application may need to be extended to 5-10 minutes.

The simple staining solutions mentioned above will reveal, upon examination with the oil immersion objective, the characteristic shape and arrangement of bacterial cells. Another method of examination, called the *negative stain*, is also useful for the same purpose. For this add a *loopful* of black or blue India ink to the drop of culture on a slide *before drying* and spread out in a thin film. Upon examination, the bacterial cell will appear unstained, clearly outlined against the dark background.

The Gram stain is a differential stain often used in bacteriology. The staining technique, which is given in the books listed above and on page 304 of this issue, employs alkaline crystal violet as the primary stain with iodine as a mordant. Following decolorization with alcohol, the counter stain of safranin is applied. Those cells retaining the crystal violet are called Gram positive, and those which lose the violet during decolorization and take up the red stain are called Gram negative. The Gram reaction of a species is of taxonomic importance and also correlates with other properties. For example, Gram positive organisms are more susceptible to the action of penicillin than Gram negative types. For correct results the culture should be not more than 24 hours old, if it will grow within that period, and the smear should be thin. The critical step is the decolorization with alcohol.

For examination of stained smears, place slide on stage of microscope, and, turning head to side, carefully lower oil immersion objective until it touches drop of oil which has been placed on *top* of stained smear. Then, using more illumination than for wet mounts, focus *upwards* slowly until image is obtained. The usual fault with smear preparations is that the smear is too thick. This is ordinarily not true of mounts from liquids, but suspensions prepared from colonies should not be "milky" before drying. Bacterial cells will be stained solidly by this procedure, but spores will be stained in outline only and center will be clear (Figure 1).

The oil must be removed from tip of objective each day before the microscope is placed in storage cabinet. Use lens paper for this, and if oil is sticky use a *small* (to prevent dissolving lens cement) amount of xylol on lens paper. A satisfactory substitute (water is not) for the cedar oil commonly supplied for this purpose may be made by adding 28.0 ml. of

Figure 1. Vegetative cells and spores of rod shaped bacterium.

alpha bromnaphthalene to 100 ml. of light weight mineral oil. This oil does not become sticky and is very satisfactory for routine use.

Transfer of Cultures

Fagle and McClung (1960) have described a variety of devices for inoculating bacterial culture solutions, but perhaps a few pointers

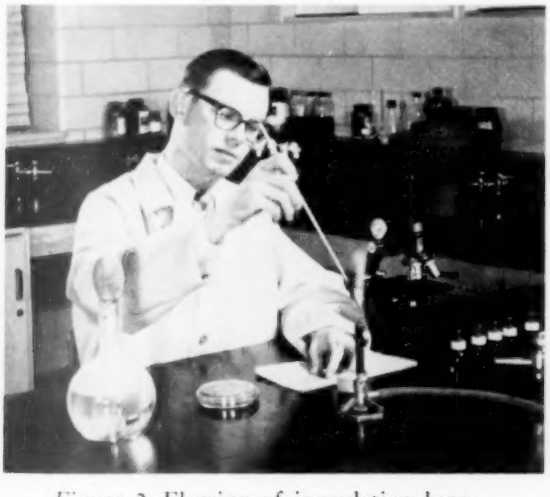


Figure 2. Flaming of inoculation loop.

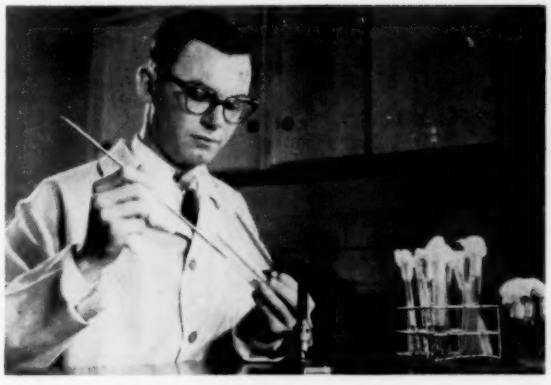


Figure 3. Method of holding tubes, plugs, and inoculation needle during transfer of cultures.

should be included concerning use of them. The wire of the inoculation loop or needle should always be heated to redness in a bunsen flame *before* and *after* use (Figure 2). It may be cooled by holding momentarily in air or, for agar cultures, by touching spot of agar where there are no colonies. To transfer from one tube to another, hold tubes as if they are extension of fingers in one hand, and with the other hand which holds the inoculation loop remove cotton plugs by holding between the fingers with the back side of the hand (Figure 3). Sterilize mouth of tubes or other containers by passing through a bunsen flame after plugs are removed. For pipettes (usually a small plug of cotton is inserted in mouth end and flamed so that no cotton protrudes beyond tip), use the forefinger (not the *thumb*) to control the level of liquid (Figures 4 and 5). After use, pipettes should be discarded in a tall jar or long tray of disinfectant.

Figure 4. Pipetting technique for aseptic transfer of liquids. Note bottle cap held between fingers.

Figure 5. Delivery of liquid to petri plate by pipette. For photographic purposes the plate, which should remain on table, is being held towards camera to show method of holding cover.

Preparation and Sterilization of Glassware

For most laboratory experiments the dry glassware should be sterilized before use, though in many short term experiments absolute sterility is not necessary. For all experiments, however, the glassware should be very clean. After use and before washing, glassware containing cultures should be sterilized either in steam (autoclave or pressure cooker) heat or thoroughly boiled in water. The glassware should then be washed using any good household soap or detergent and then be *thoroughly* rinsed. Good rinsing is particularly important if detergents are used, for otherwise a thin film of detergent may inhibit bacterial growth when the glassware is subsequently used. It is better to dry the glassware by rapid evaporation (place in oven at low heat or in some other area where the rinse water will evaporate quickly) rather than wiping with cloth.

Although sterilization of dry glassware is not absolutely required for tubes or flasks which are to receive media to be sterilized by steam heat, presterilization of the dry glassware will reduce the load of bacterial contamination to be killed when the medium is sterilized. Thus this is recommended particularly when there may be some doubts concerning the adequacy of the sterilization process for the media. This sterilization is usually accomplished by heating in an oven (cook stove with regulator is satisfactory) for at least one, and preferably two, hours at 180°C. or 356°F. The necks of tubes and flasks should be plugged with cotton before sterilization. Pipettes should be sterilized in long metal cans made especially for this purpose. Acceptable substitutes include cylindrical cardboard mailing tubes plugged with cotton at both ends. Pipettes may also be wrapped in groups in paper or metal foil. Special single service, thin, paper tubes have recently become available for this purpose.

A supply of sterile glass petri dishes is essential for many experiments in bacteriology unless pre-sterilized single service plastic plates are employed. To avoid the necessity of sterilization of the dishes immediately prior to use, it is recommended that the plates be sterilized in cans such as one pound coffee cans or after wrapping in newspaper with scotch tape to make packet units secure, in unit packages of four to six plates. This affords protection against dust contamination after sterilization. Although other plates are satisfactory, the 15 x 100 mm. assay plate with thick glass and flat bottom, available as Pyrex #3162 from most supply houses, is recommended if a new supply is to be purchased. These are used with glass (Pyrex #3160) or metal tops (consult catalogue of Baltimore Biological Laboratory) (Figure 6). Petri dishes are useful in dilution plate experiments for enumeration of bacteria in soil, water, milk, foods, etc., for pure culture isolation, for many experiments on effects of environment on bacteria, and for many other purposes.

Figure 6. Glass and metal topped petri dishes.

Purchase of Bacteriological Media

Culture media (singular, medium) for bacteria are of various types, and the choice will

depend on the purpose intended. Some are used simply to grow cultures for microscopic examination or other purposes while others will reveal certain metabolic patterns following growth. Space will not permit a full discussion of this topic, and those who need additional information should refer to Chapters II and VII of the *Manual of Microbiological Methods* and to the section of methods in the books by Skerman or Tanner.

The most convenient way of preparing many of the media used for cultivation of bacteria is to use the *dehydrated media* prepared by one of the commercial compaines. The two largest companies which prepare media are Difco Laboratories, 920 Henry Street, Detroit 1, Michigan, and Baltimore Biological Laboratory, 2201 Aisquith Street, Baltimore 18, Maryland. Products of both of these companies are more easily obtained from one of the general supply houses rather than the company directly. As indicated elsewhere, both of these companies will send on request complimentary copies of catalogues and price lists.

To use dehydrated media, the correct amount, usually indicated on the bottle, of the dehydrated powder is added to water, the powder dissolved by heat, and dispensed to appropriate tubes, flasks, or bottles, and sterilized. The cost of dehydrated media is greater than the cost of the ingredients which may be purchased separately, but, in addition to the convenience of time saving, these products offer the advantage of uniformity and thus are recommended when funds permit. Similarly, the companies mentioned and others prepare pre-sterilized media in screw cap bottles or tubes, and while these are still more expensive, they may be preferred in laboratories without sterilization facilities.

Other companies which sell media ingredients or pre-sterilized media include Albimi Laboratories, 16 Clinton Street, Brooklyn 2, N. Y.; Case Laboratories, Inc., 515 North Halsted Street, Chicago 22, Illinois; Hyland Laboratories, 4501 Colorado Blvd., P. O. Box 39672, Los Angeles 39, California; and Media, Inc., 89 Lincoln Park, Newark 2, New Jersey. As indicated above, most of the general supply houses sell the products of these companies as do certain of the biological supply houses, such as Carolina Biology Supply Co., Elon College, North Carolina; or General Biological Supply House, Inc., 8200 South Hoyne Ave., Chicago 20, Illinois. For media ingredients, usually one-quarter pound quantities are sufficient for experiments for one year. Agar, used in 1.5 -2.0% concentration for solidification of nutritive media, may often be purchased in local drug stores.

Figure 7. Removing baskets of tubed media from autoclave.

Preparation of Media

For routine cultivation of many bacteria two very useful media are nutrient broth (liquid) and nutrient agar (liquefiable solid). The formula for nutrient broth includes 0.5% peptone and 0.3% beef extract. The appropriate amounts of the dry peptone powder and the sticky beef extract are weighed out and dissolved in tap water. Distilled water is often listed for this medium but is unnecessary. The ingredients are dissolved by gentle heat and the pH adjusted to 7.0 (by addition of 1N HCl or NaOH) if colorimetric pH indicators indicate necessity of change. If tested with brom-thymol-blue, the indicator should be grass green in color. If agar is desired, add 1.5 to 2.0 grams per 100 ml. of medium.

If peptone and beef extract are not available, do not abandon attempts to cultivate bacteria for satisfactory substitutes are easily available! Try one of the formulae suggested by Sister Paulinus (1960) or one of your own from materials available to you. Additional suggestions: (1) make boiled infusion of 250 grams of hamburger in a liter of water, filter to remove meat particles, cool and remove fat, adjust pH. (2) Add 250 grams of rich soil to one liter of water, boil gently, strain, filter, and adjust pH if necessary. (3) Add 5 grams of yeast cake to 500 ml of water, boil gently,

filter, and adjust pH if necessary. (4) Steep 100 grams of hay or dried grass in one liter of water, filter, and adjust pH. The use of these as the nutritive base medium with addition of agar should, with simple air exposure plates, convince the most skeptical that bacteria can be cultivated on inexpensive materials.

Sterilization of Media

Most of the media used in bacteriology should be sterilized either in a steam autoclave as shown in Figure 7 or in a pressure cooker. The time required is usually 15-20 minutes after the pressure reaches 15 pounds and the temperature should be about 240°F. A cardinal rule to remember is to let the pressure in the autoclave or cooker return to zero *before* removing the sterilized media. To force the pressure down, by opening the steam escape valve, will cause the superheated media to boil over. *Never* shake or swirl a flask or bottle of agar media immediately upon removal from sterilizer as this will also cause the medium to boil over and the burn is very painful. The media must be sterilized the same day that it is prepared, preferably within 2 hours.

Storage of Media

The general rule, unless screw capped tubes or bottles are employed as the container, regarding media storage, is to make and sterilize only the media needed within the next week or ten days. Longer storage will bring evaporation problems unless refrigeration space is available. Prolonged storage under refrigeration sometimes involves contamination by mites, which travel through cotton plugs, or molds. Media protected from evaporation by tightly closed screw caps will last practically indefinitely. For these items usually the screw cap should be loosened one-quarter turn before sterilization and tightened afterwards.

Incubation of Bacterial Cultures

Many bacteria, certainly all that would be needed in elementary biology, grow well at ordinary room temperature (28-30° C) within 48-72 hours. For some experiments the directions may suggest incubation at 37°C but often a substitution of organisms may avoid the necessity of provision of this special incubation temperature. The list of reference articles, however, includes suggestions on adapting old refrigerators for this purpose. For many experiments the exact control of temperature is not critical. Here is another project for ingenious students who have shop facilities available.

Agar plates should be inverted agar side up during incubation. This will prevent the water of syneresis from forming a thin film on the surface which would permit bacteria to grow in a confluent film rather than forming discrete colonies.

Pure Culture Isolation Techniques

Often it is desired to isolate a pure culture of a bacterial species from the mixed population of a natural sample. The material to be studied may be a liquid culture or a suspension in sterile tap water of colony growth from an agar culture. A pure culture refers to a culture containing the cells of only one species. Two methods for obtaining a pure culture are recommended and the first is preferred since less glassware is involved, and it has other advantages.

Method 1: Pour nutrient agar plates and allow medium to harden. It will be better if

Figure 8. Draining inoculation loop within culture tube.

the plates are allowed to dry for a small number of hours after pouring. They should be inverted during this time. Charge the inoculation loop by dipping in liquid culture or suspension and drain out the liquid from the loop by touching inside of culture tube above the level of the liquid (Figure 8). Then make parallel streaks, without cutting into agar, across about one third of agar surface (Figure 9; in this photograph the petri dish lid has been raised more than it should be to permit photograph to show relation of loop to agar surface). Flame the loop, cool it by touching agar, and repeat streaks over one half of remaining area, taking care to streak across a portion of the area first inoculated. Flame needle again and repeat process with remaining area on plate. Isolated colonies should appear on one of the sectors following incubation. Such colonies should be emulsified in sterile tap water and a new plate streaked and this, on incubation, should produce colonies of uniform morphology.

Method 2: Using tubes or bottles of size to contain about 20 ml. of medium, dispense, and sterilize several such tubes. When needed, liquefy the agar by placing in pan of water which is heated to boiling point. Allow three tubes per culture to be studied to cool in a pan of water to 40-42°C. Use sterilized inoculation loop to transfer one loopful from culture suspension to tube 1 while agar is still liquid. Flame needle and lay it on desk. Rotate inoculated tube between palms of hands and transfer two loopsful from it to tube 2. Repeat process given above, transferring one loopful from tube 2 to tube 3. Before the agar column cools and solidifies, pour contents of each tube, after flaming tube lip, into a separate sterile petri dish. Upon incubation both surface and subsurface colonies should appear. These may appear to be different, even with a pure culture, for the subsurface colonies are usually either lens shaped or irregular, wooley balls, whereas the morphology of surface colonies can be quite varied with different species.

From the plates prepared by these methods, pure cultures may be established by transferring from an isolated colony to a tube of broth or agar slant appropriate to the type being studied. By these methods it is not difficult to isolate, as needed, bacteria for class exercises or special projects as Sister Paulinus (1960) has suggested.

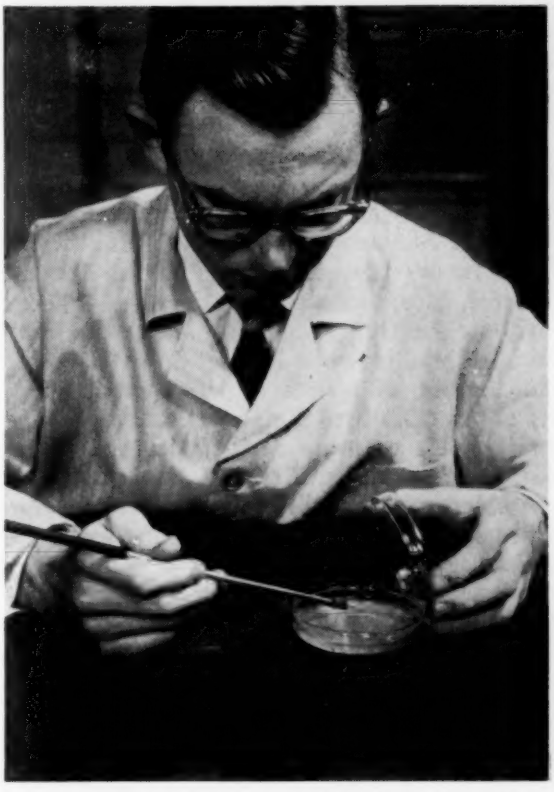


Figure 9. Technique for streaking agar plate. The plate cover is ordinarily held closer to bottom of dish.

How to Identify Bacteria

At the outset it should be stated that there are no "How to Know Bacteria" books of the same type that exist for some larger plants and animals. Bacteria may not be identified by morphological features alone, and the keys for taxonomic purposes appear at first glance to be a bit complicated for the beginner. As a matter of fact, they are complicated for the advanced student in bacterial taxonomy who may wish to consider all the possibilities in tracing the identity of a newly isolated culture. The novice is not likely, however, to need to consider *every* previously described species! With this thought in mind a few suggestions will be offered which may be of some aid to some, though most certainly not all, taxonomic problems relating to bacteria. For advice on other microorganisms, reference

should be made to some of the titles in the Reference Book List.

The "standard" American book on the classification of bacteria is *Bergey's Manual of Determinative Bacteriology* of which the 7th edition was published in 1957 by Williams and Wilkins. Skerman has presented a very useful key which will lead you to the genera in *Bergey's Manual*, but the key necessarily includes many more groups than need to be considered. Most of the types which the beginner will isolate from water, soil, air, etc., will probably be included in a small list. The following list of genera of the bacteria most likely to be isolated may be used as a preliminary guide to more detailed descriptions in Skerman's book and *Bergey's Manual*. All are aerobic, and most will grow at room temperature (some may do better at 37°C) on nutrient agar.

Gram positive rods producing spores: *Bacillus*.

Gram positive rods without spores: *Corynebacterium*, *Kurthia*, *Microbacterium* and *Mycobacterium*.

Gram negative short rods without spores: *Achromobacter*, *Aerobacter*, *Aeromonas*, *Agrobacterium*, *Alcaligenes*, *Chromobacterium*, *Erwinia*, *Escherichia*, *Flavobacterium*, *Proteus*, *Pseudomonas*, *Serratia*, and *Xanthomonas*.

Gram positive cocci: *Aerococcus*, *Gaffkya*, *Micrococcus*, *Sarcina*, *Staphylococcus*, and *Streptococcus*.

Gram negative cocci: *Neisseria*.

Gram positive filaments producing chains of conidia from aerial hyphae: *Streptomyces*.

Quantitative Plating

For most purposes at the introductory level, the method of choice for determination of numbers of bacteria in a given sample is by plating appropriate dilutions of the sample. This involves the addition of a measured amount, usually 1.0 ml., of the dilution of the sample to the bottom half of a sterile petri dish. Approximately 15 ml. of appropriate sterile agar are then poured into the dish, and the sample mixed with the agar by gentle rotation of the dish. The total number of colonies will be determined following incubation on the assumption that each living cell will give rise to a colony. Since most samples may contain a large number of cells per unit volume or weight, it is necessary to dilute the sample in order that the colony count attained will lie between 30 and 300 per plate.

Tap water is satisfactory for the diluting fluid, and the appropriate amount should be sterilized in tubes or screw capped bottles. The size of the initial sample depends on the nature of the sample. For milk or other fluids in which the population can be assumed to be evenly distributed, the initial sample of 1.0 ml. is usually added to a 9.0 ml. or 99 ml. dilution blank. With soil or other samples that are not homogenous, the initial sample would usually be larger. A good example would be to add 10 gr. of soil to a 90 ml. dilution blank or 50 gr. to 450 ml. For the purposes of these samplings, it may be assumed that 1.0 gr. will displace 1.0 ml.

If counts of any quantitative value are to be obtained, it is necessary that both the laboratory work and the calculations be done accurately. Since some samples may require high dilutions, it may be desirable to indicate briefly how these are attained. This procedure is usually accomplished by a series of steps with dilutions factors of 1-10 or 1-100 rather than, for example, adding 1 ml. of sample to 99,999 ml. of diluting fluid to attain a 1-100,000 final dilution. The latter incorrect method is not practical for at least two reasons: (1) difficulty of finding container large enought to hold 100,000 ml. which can be sterilized, and (2) difficulty of mixing small sample and large volume of diluting fluid. The more practical approach might be to use this series of dilutions.

1 ml. (or gr.) of sample + 9.0 ml. diluting fluid = 10 ml. (1-10 dilution).
1 ml. (1-10) + 9.0 ml. diluting fluid = 10 ml. (1-100 dilution).
1 ml. (1-100) + 9.0 ml. diluting fluid = 10 ml. (1-1,000 dilution).
1 ml. (1-1,000) + 9.0 ml. diluting fluid = 10 ml. (1-10,000 dilution).
1 ml. (1-10,000) + 9.0 ml. diluting fluid = 10 ml. (1-100,000 dilution).

Thus 5 dilution blanks each containing 9.0 ml. of sterile diluting fluid would be needed. A different, sterile pipette is, of course, needed for each step. Another procedure for achieving the same dilution but using different volumes of diluting fluid might be as follows.

1.0 ml. of sample + 99 ml. diluting fluid = 100 ml. (1-100 dilution).

1.0 ml. (1-100) + 99 ml. diluting fluid = 100 ml. (1-10,000 dilution).

1.0 ml. (1-10,000) + 9.0 ml. diluting fluid =10 ml. (1-100,000 dilution).

On some occasions it may not be desirable to use decimal dilutions. The principle illustrated above may be applied to other numbers, but be sure to do the calculations correctly as in the following examples of one step in five different problems.

A. 1 gr. of sample + 14 ml. = 15 ml. (1-15 dilution).

B. 3 gr. of sample + 12 ml. = 15 ml. (1-5 dilution).

C. 2 ml. (1-100) + 18 ml. = 20 ml. (1-1,000 dilution).

D. 1 ml. (1-30) + 7 ml. = 8 ml. (1-240 dilution).

E. 5 ml. (1-50) + 45 ml. = 50 ml. (1-500 dilution).

If samples of soil, milk, etc., are to be studied, the volume of the agar medium in the plate to which the sample is added *is not* considered in the calculations. The reason for this is that the number of colonies arising relate to the number of cells in the aliquot of the final dilution as they are trapped when the agar hardens. On the other hand, if certain concentrations of dye, antibiotic, or other chemicals are desired in the final medium the volume of the medium *is* taken into account.

A Word on Laboratory Cleanliness

Nowhere in laboratory work is it more important to take precautions against dust contamination than in a laboratory where bacteriology experiments are to be performed. For this reason, the immediate area, and the entire room for that matter, should be kept clear of dust collecting clutter! *Before* and *after* experiments are done, the table top should be wiped with a cloth (synthetic sponges deteriorate) moistened with disinfectant. A suitable disinfectant solution may be prepared as follows: Add 1 part of mercuric chloride to 2.5 parts of commercial (technical) hydrochloric acid. Prepare a small bottle of this stock solution (label: POISON) and for use as disinfectant solution, add 2.5 ml. of the stock solution to one liter of water. Color with a dye and label POISON.

For special work, in order to take extra precautions against room air contamination, a glass or plastic hood is employed. A convenient type may be built in the school shop. This consists of a rectangular box with sides and top of glass. The front side should be on rollers to permit opening so that the arms may reach into the work area. An alternate type utilizes a slanted front-top section which extends in the front to about six inches from the bottom of the hood. This area is left open across the front to permit access. In such hoods a microburner replaces the bunsen burner as an aid in avoiding excess heat.

References

Fagle, D. L. and McClung, L. S.: *Bacteriology inoculating devices useful in the biology laboratory*. The American Biology Teacher 22:337-338, 1960.

Paulinus, Sister Mary: *Experience with bacteria in high school biology*. The American Biology Teacher 22:325-332, 1960.

SAB History

The Society of American Bacteriologists was founded through the initiative of a volunteer committee consisting of A. C. Abbott, Professor of Hygiene and Bacteriology, University of Pennsylvania; Herbert W. Conn, Professor of Biology, Wesleyan University; and Edwin O. Jordan, Assistant Professor of Bacteriology, University of Chicago. The first meeting was held on December 28, 1899 at New Haven, Connecticut, during the 18th Annual Meeting of the American Society of Naturalists. There were 59 Charter Members. In 1960, the number of Active Members totaled approximately 6000 and there are 31 Local Branches.

Microbiology Teaching Aids

L. S. McCLUNG
Chairman, Committee on Education,
Society of American Bacteriologists

The present Committee on Education of the Society of American Bacteriologists was charged with preparing lists of materials relating to microbiology in introductory biology for distribution to teachers of biology. A mimeographed edition of the following lists was issued in the summer of 1959, and notice of availability was published in *The American Biology Teacher* and elsewhere. The large number of requests which soon exhausted the supply as well as a photoprint edition prompted the Committee to seek financial support for a printing which would be made available to the members of the National Association of Biology Teachers and to others on request. The Difco Laboratories, one of the Sustaining Members of the Society of American Bacteriologists, recognizing the need for this service to teachers, made available funds to permit the publication and distribution of this special issue containing revisions of the teaching aid lists and related manuscripts. The various members of the Committee have contributed to the lists which have been compiled and edited by the Chairman of the Committee.

Perhaps it would be desirable to comment briefly on the intent of these materials. Information available to the Committee reveals that, in the sample of over 500 teacher records studied, less than one third of the teachers of high school biology have included a course in bacteriology or microbiology in their college program of training. It appeared, therefore, that teaching aid lists might be of value. Recognizing that many teachers and others have published accounts of their laboratory experiences or made suggestions on which microbiology experiments could be based, a list (Part I) was prepared of these widely scattered articles. It may surprise many that college teachers as early as 1903 published suggestions for high school experiments. Since the very good articles appearing in *Scientific American* were of a different nature, and since this journal is becoming widely available in most school libraries, this list (Part II) is separated from the material in Part I. Teachers who desire more advanced treatment of a topic may find useful the list of reference books on microbiology (Part III). This includes, in addition to the textbooks and monographs on various aspects of microbiology, sections giving titles of biographies, juvenile literature, and books for general reading. For schools which have funds to permit extensive library purchases, the list may serve as a guide for selection of titles for special subject areas; others may find the list of value when requesting material by interlibrary loans.

Audio-visual aids may at times be needed to supplement class discussions or to substitute for laboratory experiments where facilities do do not permit each student the opportunity for first-hand experience. In the opinion of the Committee, the available film strips are either too elementary or are lacking in quality, but fortunately a number of good, recent films are available for those who desire to use them. An attempt is made in Part IV to list a variety of types so that a choice may be made of the film(s) most suitable for a particular need.

Recognizing that many schools are now beginning to purchase equipment and supplies for experiments in microbiology and that the materials which may be needed are not always commonly available from the supply companies which normally service high schools, in Part V an attempt has been made to list representative firms from which the usual materials are available. It should be emphasized that this list is intended to be representative only and that other companies may have similar or equal items.

The Committee has frequent requests from teachers and from students for suggestions for laboratory experiments for class use and for student projects. A future project of the Committee, or some members of it, concerns the development of a handbook in which detailed instructions may be outlined for such experiments. It is believed, however, that the suggestions developed in Part VI may be of value to those who have facilities and some background of experience. It is evident, however,

that these factors vary so widely from school to school that a uniform set of experiments which may be ideal in one situation is of little value in another. Therefore, as indicated, this is purposely a list of ideas on which experiments and projects may be developed depending on time and facilities. Those who use these ideas may wish to formulate definite directions for experiments along the lines of the samples presented.

Part I

Reference Articles on Use of Microorganisms in Introductory Biology

The selected list given below includes references to the use and importance of bacteria and viruses, and in some instances other microorganisms, in introductory biology. It is hoped that the experiences of students and teachers as reported in these articles will stimulate other teachers of introductory biology to use microorganisms as experimental materials for demonstration of biological phenomena. Specific suggestions are given elsewhere in this issue of *The American Biology Teacher* concerning the laboratory techniques necessary for experiments similar to those reported in the references listed below. It is evident from the articles of Frost and Hastings that as early as 1903 suggestions were made concerning bacteriology in high school biology, though apparently the trend was not established until the 1940's, and a marked increase in number of articles is noted in the 1950's. This list does not include articles from the weekly general news magazines, *Science Newsletter*, *Science Digest*, *Reader's Digest*, *Saturday Evening Post*, *Scientific American* (see pp. 356-359 of this issue), and *Science World*. Pamphlet material on bacteria is also available from many industrial companies and from some of the life insurance companies.

1903

FROST, W. D., and HASTINGS, E. G. Bacteriology for high schools. I. Journal of Applied Microscopy and Laboratory Methods, *VI*, 2205-2208, 1903

FROST, W. D., and HASTINGS, E. G. Bacteriology for high schools. II. J. of Applied Microscopy and Laboratory Methods, *VI*, 2270-2273, 1903

FROST, W. D., and HASTINGS, E. G. Bacteriology for high schools. III. Microscopical examination of bacteria. J. Applied Microscopy and Laboratory Methods, *VI*, 2383-2385, 1903

FROST, W. D., and HASTINGS, E. G. Bacteriology for high schools. IV. Microscopic examination of bacteria. J. Applied Microscopy and Laboratory Methods, *VI*, 2426-2428, 1903

FROST, W. D., and HASTINGS, E. G. Bacteriology for high schools. V. Microscopical examination of bacteria. J. Applied Microscopy and Laboratory Methods, *VI*, 2522-2524, 1903

1939

HILLIARD, C. M. Suggestions for laboratory work in bacteriology for high school students. American Biology Teacher, *1*, 134-136, 1938-39 (March, 1939)

1940

VANCE, B. B. An introductory series of problems in practical bacteriology for the high school biology course. American Biology Teacher, *2*, 83-86, 1939-40 (January, 1940)

1945

BOUSA, L. M. Bacteria. Grade Teacher, *63*, 58- , 1945 (October)

TURNER, C. E., and LYTLE, E. L. The nature of bacteria. School Health Monograph No. 7, 1945. Metropolitan Life Insurance Company, New York (out of print)

1946

GERBACH, J. B. Class demonstration of the effects of heavy metals upon bacterial growth. American Biology Teacher, *9*, 9-12, 1946-47 (Oct., 1946)

HUTCHINGS, L. M. Microbes. Cornell Rural School Leaflet, Children's Number, *40*, #3, 32 pp. New York State College of Agriculture at Cornell University, Ithaca, New York, 1946-47 (out of print)

IAMS, M. B. An improvised incubator for bacteria. American Biology Teacher, *9*, 7-8, 1946 (October)

LACROIX, D. S. Use the home pressure cooker to sterilize your culture media. American Biology Teacher, *8*, 88, 1946 (January)

1948

HERRICK, J. A. Koch's postulates as a simple laboratory exercise in biology. Science Education, *32*, 34-35, 1948 (February)

1949

JONES, A. W. A simple growth-rate experiment for an elementary course in bacteriology. American Biology Teacher, *11*, 164-165, 1949 (November)

LACROIX, D. S. Yeast experiments for the high school students. American Biology Teacher, *11*, 86-87, 1949 (March)

1950

BRYAN, C. A. Possible book and periodical contamination by bacteria and viruses. School Science and Mathematics, *50*, 58-61, 1950 (January)

JOSEPH, SISTER JOHN. Do you believe in germs? Catholic School Journal, *50*, 81-83, 1950 (March)

1951

AQUIN, SISTER M. Bacteriology project. American Biology Teacher, *13*, 10-11, 1951 (January)

BUZZETTI, B. Aids for teaching health at the senior high school level. American Biology Teacher, *13*, 110-112, 1951 (May)

1952

BRYAN, A. H. Bacteriology unit project; simple tests for antiseptic and germicidal activity. School Science and Mathematics, *52*, 364-366, 1952 (May)

HARROLD, J. P. Using the community as a laboratory. American Biology Teacher, *14*, 147-150, 1952 (October)

KNIGHT, C. A. Viruses. Science Teacher, *19*, 11-14, 1952 (February)

PATTERSON, SISTER M. T. "Painting" with bacteria. American Biology Teacher, *14*, 30-31, 1952 (February)

1953

DRAPHIN, H. Antibiotic display. Science Teacher, *20*, 179-180, 1953 (September)

FORBES, M. Materials and methods for the study of some common molds in high school biology. Master's Thesis, 1953, Iowa State Teachers College, Cedar Falls, Iowa

RZEPKA, L. A microscope for the junior scientist. School Science and Mathematics, *53*, 24-26, 1953 (January)

THOMPSON, J. J. Teaching scientific method in a unit on bacterial disease. School Review, *61*, 413-420, 1953 (October)

1955

BRICE, A. T. Micro-biology in secondary classrooms. Audio-Visual Guide, *22*, 31-32, 1955 (November)

CASE, F. Germs in the band room. Music Educators Journal, *42*, 61-63, 1955 (September)

GAWADI, H. Suggested practical work for a topic on microbes, pp. 50-55. *In* Science Teaching Techniques, *IV*, published *for* Association of Teachers in Colleges and Departments of Education, etc. *by* John Murray, Albemarle Street, London, W.1, England.

MECUM, I. M. The significance of Pasteur's attempt to disprove the theory of spontantous generation. Science Teacher, *22*, 70-74, 97-99, 1955 (March)

1956

ANONYMOUS. Elementary experiments in bacteriology. Turtox Service Leaflet No. 50. 1956 (Available (gratis) from General Biological Supply House, 8200 South Hoyne Avenue, Chicago 20, Illinois)

BRYAN, W. R. The induction of cancer by a virus. Science Teacher, *23*, 97-99, 1956 (March)

FAGLE, D. L. Another use for aluminum foil. American Biology Teacher, *18*, 196-197, 1956 (October) (Directions for making petri plates from aluminum foil)

FAGLE, D. L. Handbook of bacteriology for high school teachers. Master's Thesis, 1956, Iowa State Teachers College, Cedar Falls, Iowa

LEIGHTON, J. Tissue culture. Science Teacher, *23*, 139-141, 1956 (April)

LOWE, J. M. Polio education: a live study for the classroom. Science Teacher, *23*, 239-240, 259, 1956 (September)

SHEEK, M. L. The ABC's of bacteriology. Science Teacher, *23*, 398, 1956 (December)

WOLF, F. E. An exotic aspect of bacterial spores. American Biology Teacher, *18*, 170-171, 1956 (April)

WOODRUFF, H. B. Microorganisms in everyday life. American Biology Teacher, *18*, 153-155, 1956 (April)

1957

CERNEY, J. V. Training-room germ carriers. Scholastic Coach, *27*, 30, 40, 1957 (November)

GOSS, R. C., and HOUGH, R. R. Why not make a mould garden? Turtox News *35*, 114-116, 1957 (May)

POLLOCK, F. J. The stunted planaria. Tomorrow's Scientists, *2*, #2, 1, 6-7, 1957 (November)

WEINBERGER, R. Hands as agents of transfer of bacteria. High Points, *39*, 72, 1957 (May)

WEINBERGER, R. Antibiotic substances of bacteria. High Points, *39*, 43, 1957 (October)

1958

ANONYMOUS. Advanced experiments in bacteriology. Turtox Service Leaflet No. 52. 1958 (Available gratis from General Biological Supply House, 8200 South Hoyne Ave., Chicago 20, Illinois.

ANONYMOUS. [Making radioautographs of bacteria] Tomorrow's Scientists, *3*, #2 (Oct.), 1, 4, 1958.

ANONYMOUS. Bacteria's deadly enemy. Tomorrow's Scientists, *3*, #4 (Dec.), 6, 1958.

ANONYMOUS. Bacteriophage. Tomorrow's Scientists, *3*, #4, (Dec.), 4, 1958.

ANONYMOUS. Is it safe to drink? Tomorrow's Scientists, *3*, #4 (Dec.), 7, 1958.

ANONYMOUS. Radioautographs of yeast cells. Teaching Tomorrow's Scientists *3*, #3-4 (Nov.), 1, 1958 (Suggestions for procedure)

ANONYMOUS. Streptococci research. Tomorrow's Scientists *3*, #2 (Oct.), 7, 8, 1958.

ADLER, L. K. Simple demonstration of the bacteriostatic effect of antibiotics. Science Teacher, *25*, 216-17, 1958 (May)

ANDREWS, J. M. Unraveling the viral diseases. Tomorrow's Scientists *3*, #*4*, (Dec.), 1-2, *6*, 1958.

DIEHL, T. H., THOMAS, R. C., and CAMPBELL, J. F. From research to classroom laboratory . . . high temperature, short time pasteurization of milk. Science Teacher, *25*, 394-397, 405, 1958 (November)

FAGLE, D. L. Improvisation of bacteriological equipment. American Biology Teacher, *20*, 252, 1958 (November)

GOSS, R. C. What causes the holes in Swiss cheese? American Biology Teacher, *20*, 289-291, 1958 (December)

HARTKER, R. I., TARZWELL, C. M., and HENDERSON, C. Basic steps to teaching bioassay of water. Science Teacher, *25*, 120-136, 1958 (April)

HEINLEIN, C. L., and COOKE, W. B. Micro-fungi in the soil. Science Teacher, *25*, 20-24, 1958 (February) (Recommended for the ninth-grade student interested in mycology or in science project work.)

HEINLEIN, C. L., and GELDREICH, E. E. From research to classroom laboratory: Culturing bacteria on membrane filters. Science Teacher, *25*, 328-331, 343, 1958 (October)

HEINLEIN, C. L. and GELDREICH, E. E. Pour plate technique. Science Teacher, *25*, 16-19, 1958 (February) (Growing and counting bacteria from a water sample.)

HICKS, B. Growing more meat with antibiotics. Popular Mechanics Magazine, March, 1958.

JOHNSON, R. M. The use of bacteria in high school biology. American Biology Teacher, *20*, 41-42, 1958 (February)

LESSER, M. S. Teaching scientific methods in yeast study. A lesson plan. Science Teacher, *25*, 377-379, 381, 1958 (November)

LORANGER, F. I. Microbiologic experiments designed for high school pupils. Department of Bacteriology, University of Michigan, Ann Arbor, Michigan. 27 pp., 1958 (Available gratis from Department of Bacteriology, Medical School, University of Michigan, Ann Arbor, Mich.)

PRESSLER, A. E. Bacteria that make life possible. Science Teacher, *25*, 309-310, 1958 (October)

REPOLE, M. Nature of antibody antigen reaction. *Journal of Biology 1958, pp. 6-8.

SATIR, K. R. Aureomycin as a metabolite for certain soil microbes. *Journal of Biology 1958, pp. 32-34.

SCHWARTZ, J. The lady and the virus. *Journal of Biology 1958, pp. 31-35

STECKER, F. The bacteriophage: a cure for bacterial diseases. *Journal of Biology 1958, pp. 16-17, 21

STRONG, C. L. (ed.) (Lawrence, R. and Soloway, H.) Amateur scientist: how to cultivate harmless bacteria. Scientific American *198*, 134-136, 1958 (March) (See also: D. H. Howard, *199*, 210, 1958—September)

*Journal of Biology is published by the Biology Department of the Bronx High School of Science, 75 West 205th Street, Bronx 68, New York.

1959

ANONYMOUS. "Cancer-Less" research on cancer. Tomorrow's Scientists, *3*, #7 (March), *6*, 1959; Teaching Tomorrow's Scientists, *3*, #7-*8* (March), 1959.

ANONYMOUS. Digestion in protozoa. Tomorrow's Scientists, *3* #*5* (Jan.), 2, 1959

AMMEL, T. J. and RICE, C. W. An incubator from an old refrigerator cabinet. Science Teacher, *25*, 182-183, 1959 (April)

BAKER, A. J. Antibiotics from your backyard. Science Teacher, *26*, 331, 333, 1959 (September)

BRYAN, A. K. School band wind instrument mouthpieces may harbor countless disease germs. School Science and Mathematics, *59*, 9-15, 1959 (January)

GREEN, W. S. Visual aids for teaching bacteriology. The Science Teacher, *26*, 85-89, 1959 (March)

MARALDO, J. Enzyme isolation. **High School Science and Mathematics Journal, *1*, 29-31, 1959 (May)

MARTIN, E. Plant galls and light-sensitivity. *Journal of Biology 1959, pp. 22-23, 33

McCLUNG, L. S. Program of the Committee on Education of the Society of American Bacteriologists. American Biology Teacher, *21*, 242, 1959 (October)

PANTELL, R. DNA and heredity in bacteria. *Journal of Biology 1959, pp. 34-35

PEARLMAN, R. Pigment production in *Serratia marcescens*. *Journal of Biology 1959, pp. 3-5

RICHTER, M., and FRASER, D. The use of bacteriophages in high school biology. American Biology Teacher, *21*, 282-287, 1959 (November)

WAXMAN, J. Living insecticides. *Journal of Biology 1959, pp. 16-17

WEINBERG, E. D. Gradient agar plates. American Biology Teacher, *21*, 347-350, 1959 (December)

WEINBERG, E. D. A simple method for the demonstration of antagonism and synergism of biologically active chemicals. American Biology Teacher, *21*, 186-188, 1959 (May)

WEINSTEIN, S. Pigment production in *Serratia marcescens*. *Journal of Biology 1959, pp. 3, 44-46

No Date

ANONYMOUS. The culture and microscopy of molds. Turtox Service Leaflet No. 32.

CAMPBELL, A. H. School lessons in bacteriology. School Nature Union Pamphlet No. 98, *N. P.* (England)

GALLOWAY, L. D. Simple experiments in microbiology. School Nature Union Pamphlet No. 84, *N. P.* (England)

KRAMER, L. M. J. The child's microbial heritage, British Social Biology Council, Tavistock House South, Tavistock Square, London, W. C. I., England.

**High School Science and Mathematics Journal is published by University of Colorado, Boulder. Editor: Edwin M. Fields, Hellems Annex 318.

Part II

Papers of Microbiological Interest Published in Scientific American in Recent Years

The popular monthly magazine, *Scientific American*, of interest and value to scientists of all fields, has included numerous articles relating to microbiology. These are written by authorities who are conducting research in the area of the topic and who have the ability to summarize the laboratory results in a manner which is both informative and entertaining to others. Representative articles, with brief abstracts, for the years 1951 through 1959 are listed below. Reprints of certain of these articles may be obtained at 10 cents per copy from Department BR, Scientific American, 415 Madison Avenue, New York 17, N. Y.

1951

Vol. 184: No. 1 January, 1951

FLAGELLA by W. T. Astbury p. 20-24

"The whiplike appendages of bacteria are among the smallest living structures. They behave like small muscles; thus they are studied to reveal the basic properties of muscle and other contractile proteins."

Vol. 184: No. 2 February, 1951

WHITE BLOOD CELLS V. BACTERIA by W. Barry Wood, Jr. p. 48-52

"What Lord Lister called one of the most 'romantic' stories in the history of pathology—the investigation of the role played by phagocytes in defending man against disease—is here reviewed and brought up to date."

Vol. 184: No. 4 April, 1951

THE IMITATIVE DRUGS by Richard O. Roblin, Jr. p. 60-63

"The sulfa drugs are actually counterfeit vitamins. The discovery that molecules of similar structure compete with one another in biochemical reactions provides a tool for studying metabolism and drug action."

Vol. 184: No. 5 May, 1951

REVIVAL BY LIGHT by Albert Kelner p. 22-25

"Concerning the discovery that visible radiation can resuscitate microorganisms that have apparently been killed by ultraviolet. The phenomenon opens up a whole new study in the action of radiation on protoplasm."

Vol. 184: No. 5 May, 1951

VIRUSES by F. M. Burnet p. 43-51

"The very small microorganisms that grow only in cells are studied not only for the treatment and prevention of the infections they cause but also for themselves."

Vol. 185: No. 5 November, 1951

PURPLE BACTERIA by Roderick K. Clayton and Max Delbruck p. 68-72

"Certain microorganisms have a characteristic reaction to the presence or absence of light. They can thus be used as a means of studying the fundamental mechanism by which living things react to their environment."

1952

Vol. 186: No. 1 January, 1952

MOLDS AND MEN by Ralph Emerson p. 28-32

"The fungi exert a remarkable influence on human affairs, affecting such things as the size of a wheat crop, the control of infectious diseases, the construction of houses and even the number of Irishmen in the U. S."

Vol. 186: No. 3 March, 1952

STREAM POLLUTION by Rolf Eliassen p. 17-21

"The means to control this major threat to one of our most valuable natural resources are at hand or in development, but it will cost the U. S. some $20 billion to restore its watercourses to a reasonable purity."

Vol. 186: No. 4 April, 1952

THE PROGRESS OF ANTIBIOTICS by Kenneth B. Raper p. 49-57

"These substances have saved innumerable lives in the decade since the first penicillin treatments, and production of the drugs has become a major industry. A survey of scientific and industrial progress in the field."

Vol. 186: No. 6 June, 1952

PLANT CANCER by Armin C. Braun p. 66-72

"Crown gall is a tumerous growth in plants which is similar in many ways to animal cancers. The disease is now being intensely studied for the light it sheds on the general problem of tumor origin and formation."

Vol. 187: No. 4 October, 1952

THE DIPHTHERIA TOXIN by A. M. Pappenheimer, Jr. p. 32-36

"How can organisms too small to be seen with the naked eye kill an animal as large as man? A study of the mechanism by which the poison from one such bacillus interrupts a vital process of the living cell."

Vol. 187: No. 5 November, 1952

A NEW ERA IN POLIO RESEARCH by Joseph L. Melnick p. 26-29

"The discovery that polio virus could be grown in test tubes as well as in the spinal cords of living

monkeys was the first big break in a long campaign. Now we can hope for more rapid progress against the disease."

Vol. 186: No. 6 June, 1952

THE ERADICATION OF MALARIA by Paul F. Russell p. 22-25

"In 1945 there were 411,600 malaria cases in Italy; in the first half of 1951 there were 392, and no deaths have been reported for the past three years. DDT has made possible almost complete elimination of the disease."

Vol. 187: No. 6 December, 1952

THE USEFUL ALGAE by Francis J. Weiss p. 15-17

"Ranging from single cells to tree-like colonies, these primitive plants support a whole heirarchy of marine life and also contribute directly to the needs of man."

1953

Vol. 188: No. 2 February, 1953

THE GEOGRAPHY OF DISEASE by Jacques M. May p. 22-27

"Knowing where a disease is prevalent, and where it is not, may furnish important clues as to its cause and prevention. Presenting some of the first results of a project for mapping man's illnesses over the globe."

Vol. 188: No. 2 February, 1953

TOXOPLASMOSIS by Reginald D. Manwell and H. P. Drobeck p. 86-92

"This little-known disease is surprisingly widespread and sometimes fatal. Although it has been studied for 50 years, it is still a medical and biological enigma."

Vol. 188: No. 3 March, 1953

DISCOVERIES IN NITROGEN FIXATION by Martin D. Kamen p. 38-42

"A lucky experimental accident and some shrewd investigation has recently brought to light a surprising multitude of microorganisms with the ability to take nitrogen from the air and fix it in useful chemicals."

Vol. 188: No. 3 March, 1953

THE EMBRYOLOGIST AND THE PROTOZOON by Paul B. Weisz p. 76-82

"A tale of the biological laboratory. A one-celled animal is cut to pieces with curious results that clarify how a fertilized egg gives rise to the specialized tissues of a many-celled organism."

Vol. 188: No. 4 April, 1953

THE INFLUENZA VIRUS by Macfarlane Burnet p. 27-31

"The organism that causes the disease which periodically sweeps through large sections of the human species is curiously changeable. It is this property that makes it difficult to prevent the epidemics with vaccines."

Vol. 188: No. 5 May, 1953

THE MULTIPLICATION OF VIRUSES by Gunther S. Stent p. 36-39

"In a series of marvelously ingenious experiments with radioactive tracers, biologists are laying bare the steps by which these viruses enter a bacterium and, in 24 minutes, manufacture 200 replicas of themselves."

Vol. 188: No. 6 June, 1953

A VERSATILE VIRUS by Karl Maramorosch p. 78-86

"China asters are affected with a searing disease called aster yellows, caused by an organism which is on the borderline between plant and animal viruses. Its study is linking up the two fields, once separated."

Vol. 189: No. 1 July, 1953

GAMMA GLOBULIN IN POLIO by William McD. Hammon p. 25-29

"How a fraction of blood plasma offers short-term immunization against the paralytic disease. Unfortunately the utility of the substance is limited by its short supply, and medicine still awaits a successful vaccine."

Vol. 189: No. 4 October, 1953

ALGAE AS FOOD by Harold W. Milner p. 31-35

"Under controlled conditions single-celled plants can be grown to yield proteins and fats at rates that far outdo agricultural yields per acre. Whether such foods will be both economic and palatable is a question."

Vol. 189: No. 4 October, 1953

EVOLUTION OBSERVED by Francis J. Ryan p. 78-82

"Because energetically multiplying bacteria can crowd into a few years generations equal to a million years of man's development, evolution may be observed. It brings firm confirmation to the Darwinian Thesis."

Vol. 189: No. 6 December, 1953

VIRUSES WITHIN CELLS by Joseph L. Melnick p. 38-41

"A recently developed technique for slicing tissue into ultrathin sections makes it possible for the electron microscope to look into a cell infected with virus and to photograph some of the stages of viral reproduction."

1954

Vol. 190: No. 2 February, 1954

THE RABBIT PLAGUE by Frank Fenner p. 30-35

"Myxomatosis is a virus disease nearly 100 per cent fatal for the common European rabbit species. Its deliberate introduction into Australia in 1950 and more recently in Europe is viewed as a two-edged sword."

Vol. 100: No. 3 March, 1954

THE LIFE CYCLE OF A VIRUS by André Lwoff p. 34-37

"What happens in the time between the infection of a cell by a virus and the appearance of a new generation of the viruses? The evidence of bacteriophages suggests that the process involves the concept of the provirus."

Vol. 190: No. 6 June, 1954

FERTILIZATION AND ANTIBODIES by Albert Tyler p. 70-75

"Fertilization of an egg and viral invasion of a cell involve the same biological interaction—the antigen-antibody reaction. Experiments on sea-urchin eggs leads to an understanding of the basic mechanism."

Vol. 191: No. 3 July, 1954

HEAT AND LIFE by Frank H. Johnson p. 64-68

"Life is limited to the zone between the freezing and boiling points of water, where its processes are synchronized by heat sensitive enzymes."

Vol. 191: No. 5 November, 1954

HOW ANTIBODIES ARE MADE by Sir MacFarlane Burnet p. 74-78

"Antibodies have a way of sticking only to the type of molecule or microorganism against which they are developed. They illustrate the body's remarkable ability to distinguish between native and foreign material."

Vol. 191: No. 6 December, 1954

THE PHYSICS OF VIRUSES by Ernest C. Pollard p. 62-70

"What are viruses like: To answer this physical question a variety of modern physical tools have been enlisted. Among these is the cyclotron whose radiation is being used to probe into the structure of viruses."

1955

Vol. 192: No. 1 January, 1955

RICKETTSIAE by Marianna R. Bovarnich p. 74-79

"Widely known as the cause of typhus, these primitive organisms now hold the stage for other reasons. Midway between viruses and bacteria they may help solve the evolutionary puzzle presented by viruses."

Vol. 192: No. 3 March, 1955

UNKNOWN VIRUSES by George Gray p. 60-70

"Viruses usually reveal themselves through infection, but now virologists have come up with some new one for which no known diseases have been found. Some viruses are now thought to fall into kinship patterns."

Vol. 192: No. 4 April, 1955

VACCINES FOR POLIOMYELITIS by Jonas E. Salk p. 42-44

"We shall soon know the results of last year's large-scale test of the new vaccine against poliomyelitis. Some of the work leading up to the present vaccine has thrown new light on the mechanism of immunization."

Vol. 192: No. 4 April, 1955

THE T2 MYSTERY by Salvador E. Luria p. 92-98

"'Controlled sloppiness' in an experiment led to the discovery that bacterial viruses may sometimes be altered by the host in which they reproduce. The effect casts new light on the heredity of viruses and cells."

Vol. 192: No. 5 May, 1955

SECOND THOUGHT ON THE GERM THEORY by René J. Dubos p. 31-35

"When the late George Bernard Shaw mischievously suggested that 'The characteristic microbe of a disease might be a symptom instead of a cause,' he was not so far from right. It is useful to consider it as a bit of both."

Vol. 192: No. 6 June, 1955

ANTIBIOTICS AGAINST PLANT DISEASES by David Pramer p. 82-90

"A number of economically important blights and other plant diseases may soon be controlled by antibiotic sprays. The study of ways in which the drugs are taken up has revealed new facts about plant physiology."

Vol. 192: No. 6 June, 1955

THE GERM OF TUBERCULOSIS by Esmond R. Long p. 102-110

"Although the tubercle bacillus has been studied more intensively than almost any other microorganism, and although medical measures against it are increasingly effective, the reason for its virulence is a mystery."

Vol. 193: No. 1 July, 1955

THE MUTATION OF VIRUSES by C. A. Knight and Dean Fraser p. 74-78

"In 1918 and 1919 an influenza epidemic killed 25 million people. It was due to a change in the hereditary constitution of a virus. Such changes are studied as a clue to the mechanism of mutation in all living things.

1956

Vol. 194: No. 5 May, 1956

EXPERIMENTS IN PROTEIN SYNTHESIS by Ernest F. Gale p. 42-46

"Broken cells shed light on the role of nucleic acids in the making of protein."

Vol. 194: No. 6 June, 1956

REBUILDING A VIRUS by Heinz Frankel-Conrat p. 42-47

"The most primitive living organism can now be reconstructed from its components."

Vol. 195: No. 1 July, 1956

SEXUALITY IN BACTERIA by Elie L. Wolman and Francois Jacob p. 109-118

"When bacteria reproduce sexually (a rare event), they clarify the genetic process."

Vol. 195: No. 2 August, 1956

LIVING INSECTICIDES by Edward A. Steinhaus p. 96-104

"A new arsenal of selective microbes is helping farmers in their war against insects."

Vol. 195: No. 5 November, 1956

TRANSFORMED BACTERIA by Rollin D. Hotchkiss and Ester Weiss p. 48-53

"Nuclear material from one bacterial strain confers its traits on another strain."

1957

Vol. 196: No. 2 February, 1957

THE STRUCTURE OF THE INFLUENZA VIRUS by F. M. Burnet p. 37-43

"The behavior of the virus is clarified by studies of its physical and chemical nature."

Vol. 197: No. 1 July, 1957

AGAMMAGLOBULINEMIA by David Gitlin and Charles A. Janeway p. 93-104

"It is a disease in which the blood lacks gamma globulin to fight bacterial infection."

Vol. 197: No. 2 August, 1957

SINGLE HUMAN CELLS IN VITRO by Theodore T. Puck p. 91-100

"A new technique devised by the author permits them to develop and multiply."

Vol. 197: No. 4 October, 1957

THE SPECIFICITY OF ANTIBODIES by S. J. Singer p. 99-106

"How is it that an antibody molecule joins with only one kind of antigen molecule?"

Vol. 197: No. 6 December, 1957

TOOTH DECAY by Reidar F. Sognnaes p. 109-116

"Tooth enamel is not inert but is a living tissue which can be invaded by bacteria."

1958

Vol. 198: No. 2 February, 1958

THE METABOLISM OF RUMINANTS by Terence A. Rogers p. 34

"Cows can digest grass by virtue of four stomachs and cooperative microorganisms."

Vol. 199: No. 1 July, 1958

PREDATORY FUNGI by Joseph J. Maio p. 67-72

"Some molds are able to trap and devour small animals which reside in the soil."

Vol. 199: No. 2 August, 1958

POISONOUS TIDES by S. H. Hunter and John J. A. McLaughlin p. 92-98

"The explosive multiplication of certain marine microorganisms can kill fish by the millions. Studies of these outbreaks are helping to solve a fundamental problem in ecology."

Vol. 199: No. 3 September, 1958

INNOVATION IN BIOLOGY by George Wald p. 100-113

"The biologist awaits physical sciences that can cope with the phenomena of life."

Vol. 199: No. 5 November, 1958

"TRANSDUCTION" IN BACTERIA by Norton D. Zinder p. 38-43

"Genes can be transported from one bacterium to another by an infective virus."

1959

Vol. 200: No. 1 January, 1959

THE STAPYLOCOCCUS PROBLEM by Stewart Mudd p. 41-45

"A ubiquitous parasite has acquired resistance to antibiotics and is causing epidemics of purulent infection in hospitals. Control calls for renewed research and a return to aseptic and antiseptic routines."

Vol. 200: No. 2 February, 1959

ENTEROVIRUSES by Joseph L. Melnick p. 89-97

"It is now apparent that the three viruses which cause polio belong to a family of more than 50 viruses which ordinarily dwell in the human alimentary tract without causing disease."

Vol. 200: No. 6 June, 1959

BEER by Anthony H. Rose p. 90-100

"Man has made this exquisite beverage for 6,000 years, but it is only in the past century that he has begun to understand the subtle enzyme chemistry and microbiology of the process."

SAB Annual Meetings

The Society of American Bacteriologists invites all teachers of science, high school students, and undergraduate college students who are especially interested in microbiology to register without charge at its 1960 Annual Meeting in Philadelphia. The special registration will be available on Wednesday, May 4, and the special badge will entitle the wearer to visit the Commercial and Scientific Exhibits and to participate in a special late afternoon reception which will be followed by a program on the opportunities in microbiology. Registration will be in the Bellevue-Stratford Hotel.

Confirmed future meeting dates of the Society of American Bacteriologists are:

1960—Philadelphia, May 1-5
1961—Chicago, April 23-27
1962—Kansas City, May 7-12
1963—Cleveland, Early May
1964—Washington, D. C., May 3-7

Part III

Selected List of Reference Books in Microbiology

This is not an exhaustive list of all books that have been published about microorganisms and microbiology but rather a selective list of books printed in English, arranged by the general topics indicated below. It was developed as an aid to students and teachers in schools without an extensive library to permit a possible selection of a title within a given broad subject area to purchase or obtain on inter-library loan. It would be assumed that most or all of the titles are available in the biology or medical libraries of most large universities, the Library of Congress and/or National Library of Medicine. Some of the titles, indicated by *, are considered to be especially suitable for high school libraries (in most subject sections only one title is so marked and in some cases there was little reason for choice) and a few are more elementary—these are indicated by **.

The titles are arranged in the following broad subject areas:

Page	Topic
360	Biographies and General Reading
362	Juvenile Literature
362	Algae
362	Animal Care
363	Antibiotics
363	Chemotherapy, Pathogenicity, Virulence, Toxin Production
363	Cytology
363	Determinative (Ecology, Taxonomy, Special Groups)
364	Dictionary
364	Food and Dairy Bacteriology
364	Genetics, Adaptation, Drug Resistance
364	History
364	Immunology
365	Industrial Fermentation
365	Insect Microbiology
365	Laboratory Methods
365	Microscopy—Elementary —Advanced Level —Electron
366	Molds, Yeasts, and Filamentous Bacteria
366	Physiology (Growth, Death, Chemical Relationships)
367	Plant Diseases
367	Protozoology
368	Sanitary (Water and Sewage) Bacteriology
368	Soil, Petroleum, and Marine Microbiology
368	Tissue Culture and Germ-free Techniques
368	Virology (Bacteriophages and Plant and Animal Viruses)
369	Textbooks—General —Introductory or Non-Technical —Medical —Veterinary

Biographies and General Reading

ALLEN, P. W. The story of microbes, 454 p., The Bookmill Co., Knoxville, Tenn., 1932

ANONYMOUS Our smallest servants, 32 p., Chas. Pfizer and Co., Inc., 11 Bartlett Street, Brooklyn 6, N. Y., 1955

*BARON, A. L. Man against germs, 320 p., E. P. Dutton and Co., Inc., New York, 1957

*BAYNE-JONES, S. Man and microbes, 128 p., Williams and Wilkins, Baltimore, Md., 1932

**BEN MEYR, B. Your germs and mine: the story of good and bad microbes, 389 p., Doubleday, Doran and Company, Inc., Garden City, N. Y., 1934

**BENZ, F. Pasteur, knight of the laboratory, 232 p., Dodd, Mead and Co., 1940

*BIGGER, J. W. Man against microbes, 304 p., Macmillan Co., New York, 1939

*BLUEMEL, E. Florence Sabin: Colorado woman of the century, 238 p., Univ. Colorado Press, Boulder, Colorado, 1959

*BREAN, H. The clock strikes thirteen, 211 p., Morrow, New York, 1952

*BURNET, F. M. Natural history of infectious diseases, 2nd ed., 356 pp., Cambridge University Press, N. Y., 1953

*BURNET, F. M. Viruses and man, 2nd ed., 197 p., Penguin Books, Baltimore, Md., 1955

*CALKINS, G. N. The smallest living things; life as revealed by the microscope, 135 p., University Society, New York, 1935

*CAMERON, H. C. Joseph Lister: the friend of man, 180 p., Wm. Heinemann Medical Books, Ltd., London, 1948

**COMPTON, P. The genius of Louis Pasteur, 361 p., Macmillan Co., New York, 1932

*DE KRUIF, P. Microbe hunters, 368 p., Harcourt, Brace and Co., N. Y., 1941

*DOBELL, C. Antony van Leeuwenhoek and his "little animals". . . , 435 p., Harcourt, Brace and Co., N. Y., 1932

**DOLAN, E. F. Pasteur and the invisible giants, 214 p., Dodd, Mead and Co., N. Y., 1958

**DOORLY, E. The microbe man; a life of Pasteur for children, 159 p., Wm. Heinemann Ltd., 1951

*DREW, J. G. Man, microbe and malady, 209 p., Penguin Books, Harmondsworth, Middlesex, England, 1950

*DUBOS, R. Louis Pasteur: Free lance of science, 418 p., Little, Brown and Co., Boston, 1950

*DUBOS, R. Mirage of health: utopias, progress, and biological change (vol. 22 of World Perspectives), 236 p., Harper and Bros. Publishers, New York, 1959

*DUBOS, R. J. and DUBOS, J. The white plague; tuberculosis, man, and society, Little, Brown and Co., Boston, 1952

*ECKSTEIN, G. Noguchi, 419 p., Harper and Bros., N. Y., 1931

*FISCHER, M. William B. Wherry, bacteriologist, 293 p., Chas. C. Thomas, Springfield, Illinois, 1938

*FLEXNER, S., and FLEXNER, J. T. William Henry Welch and the heroic age of American medicine, 539 p., The Viking Press, N. Y., 1941

**FOX, R. Great men of medicine, 240 p., Random House, N. Y., 1947

**FOX, R. Milestones of medicine, 237 p., Random House, N. Y., 1950

*GIBSON, J. M. Soldier in white; the life of General George Miller Sternberg, 277 p., Duke University Press, Durham, N. C., 1958

**GRANT, M. P. Louis Pasteur: fighting hero of science, 220 p., Whittlesey House, 1959

*GRANT, M. P. Wonder world of microbes, 157 p., Whittlesey, 1956

*GROONOWICZ, A. Bela Schick and the world of children, 216 p., Abelard-Schumann, N. Y., 1954

*HEMMING, J. Mankind against the killers, 231 p., Longmans, Green, N. Y., 1956

*KAMP, A. F., LA RIVIERE, J. W. M., and VERHOEVEN, W. Albert Jan Kluyver, his life and work, 566 p., Interscience Publishers, Inc., N. Y., 1959

*KELLY, H. A. Walter Reed and yellow fever, 355 p., The Norman, Remington Co., Baltimore, 1923

*KLUYVER, A. J., and VAN NIEL, C. B. The microbe's contribution to biology, 182 p., Harvard Univ. Press, Cambridge, Mass., 1956

*KOPELOFF, N. Man vs. microbes, 311 p., Garden City Publishing Co., Garden City, N. Y., 1930

*LEESON, J. R. Lister as I knew him, 212 p., Bailliere Tindall and Cox, London, 1927

*LUDOVICI, L. J. Fleming: discoverer of penicillin, 223 p., Indiana University Press, Bloomington, Ind., 1952

*LUDOVICI, L. J. The world of the infinitely small, explorations through the microscope, 183 p., Phoenix House, London, 1958

*MARQUARDT, M. Paul Ehrlich, 255 p., Schuman, N. Y., 1951

*MARTI-IBANEZ, F. Men, molds and history, 116 p., MD Publications, Inc., N. Y., 1958

*MAUROIS, A. (Trans by Gerard Hopkins) The life of Sir Alexander Fleming, discoverer of penicillin, 293 p., E. P. Dutton and Co., Inc., N. Y. 1959

*NICHOL, L. H. Microbes by the million, 2nd ed., 221 p., Penguin Books, Ltd., Middlesex, Eng., 1940

*OLIVER, W. W. The man who lived for tomorrow, William H. Park, 507 p., E. P. Dutton and Co., Inc., N. Y., 1941

OPARIN, A. I. (translated by Ann Synge) The origin of life on the earth, 3rd ed., 495 p., Academic Press, N. Y., 1957

**PAIN, N. Louis Pasteur, 122 p., G. P. Putnam's Sons, N. Y., 1958

PARK, W. H. and WILLIAMS, A. W. Who's who among the microbes, 302 p., Century, N. Y., 1929

**REGGIO, BROTHER EDWIN Microbe detection: a story of Louis Pasteur, 95 p., Dujario Press, Notre Dame, Indiana, 1957

*RODGERS, A. D. Irwin Frick Smith: a story of North American plant pathology, 675 p., American Philosophical Society, 1952

*ROSEBURY, T. Peace or pestilence; biological warfare and how to avoid it, 218 p., Whittlesey House, N. Y., 1949

*ROUECHE, B. Eleven blue men and other narratives of medical detection, 215 p., Little, Brown and Co., Boston, 1954

RUSH, J. H. The dawn of life, 262 p., Hanover House, Garden City, N. Y., 1957

*SCHATZ, A., and RIEDMAN, S. R. The story of microbes, 172 p., Harper, 1952

SIGEREST, H. E. Civilization and disease, 255 p., Cornell University Press, Ithaca, N. Y., 1943

*SILVERMAN, M. Magic in a bottle, 2nd ed., 386 p., Macmillan Co., N. Y., 1948

*SLAUGHTER, F. G. Immortal Magyar: Semmelweis, conquerer of childbed fever, 211 p., Henry Schuman, N. Y., 1950

*SMITH, G. Plague on us, 365 p., Oxford University Press, N. Y., 1941

*SMITH, K. M. Beyond the microscope, 143 p., Penguin Books, Baltimore, Md., 1943

*SUTHERLAND, L. Magic bullets, 139 p., Little, Brown and Co., Boston, 1956

*THOMPSON, M. The cry and the covenant, 469 p., Doubleday and Co., Inc., N. Y., 1949

*TRUAX, R. Joseph Lister, father of modern surgery, 287 p., Bobbs-Merrill Co., N. Y., 1944

*VALLERY-RADOT, P. Louis Pasteur: a great life in brief, 199 p., Alfred A. Knopf, N. Y., 1958

*VAN ITERSON, G., DEN DOOREN DE JONG, L. E., and KLUYER, J. Martinus Willem Beijerinck, his life and his work, 192 p., M. Nijhoff, the Hague, 1940

*WAKSMAN, S. A. My life with the microbes, 364 p., Simon and Schuster, N. Y., 1954

WAKSMAN, S. (ed.) Perspectives and horizons in microbiology, 220 p., Rutgers University Press, New Brunswick, N. J., 1955

*WAKSMAN, S. A. Sergei N. Winogradsky, his life and work. The story of a great bacteriologist, 150 p., Rutgers University Press, New Brunswick, N. J., 1953

*WARSHAW, L. J. Malaria, the biography of a killer, 348 p., Rinehart and Co., N. Y., 1949

*WILLIAMS, B., and EPSTEIN, S. William Crawford Gorgas: tropic fever fighter, 184 p., Julian Messner, Inc., N. Y., 1953

WILLIAMS, G. Virus hunters, 516 p., Alfred A. Knopf, N. Y., 1959

*WINSLOW, C.—E. A. The conquest of epidemic disease, 411 p., Princeton University Press, Princeton, N. J., 1944

*WOOD, L. Walter Reed: doctor in uniform, Julian Messner, N. Y., 1943

*WOOD, L. N. Louis Pasteur, 218 p., Julian Messner, Inc., N. Y., 1948

*YOST, E. American women of science, rev. ed., 233 p., J. B. Lippincott Co., Philadelphia, Pa., 1955

*ZINSSER, H. Rats, lice and history, 443 p., Little, Brown and Co., Boston, Mass., 1940

*ZINSSER, H. As I remember him: the biography of R. S., 443 p., Little, Brown and Co., Boston, Mass., 1940

Juvenile Literature (See also Microscopy, elementary)

**GRANT, M. P. Wonder world of microbes, 160 p., Whittlesey House, N. Y., 1956

**JACOBSEN, V. B., and DAINES, L. L. The adventures of Jimmy Microbe, 94 p., Reilly and Lee Co., Chicago, 1937

**JACOBSEN, V. B., and DAINES, L. L. The strange adventures of Jimmy Microbe; stories of good health habits, 95 p., Salt Lake City, 1934

**JACOBSEN, V. B., JACOBSEN, D., and DAINES, L. L. Hitch-hiking with Jimmy Microbe, 92 p., Reilly and Lee, Chicago, 1939

**LEWIS, L. First book of microbes, 64 p., Watts, N. Y., 1939

**LUDOVICI, L. J. World of the microscope, 128 p., Putnam, N. Y., 1959

**SELSAM, M. E. Microbes at work, 95 p., Wm. Morrow and Co., N. Y., 1953

**STEPHENSON, M. B. World of invisible life, 112 p., Thos. S. Rockwell Co., Chicago, 1941

**THONE, F. E. A. The microscopic world, 245 p., J. Messner, Inc., N. Y., 1940

**ZELIE, K. The fight against germs, 36 p., Row, Peterson and Co., 1941

Algae

BRUNEL, J., PRESCOTT, G. W., and TIFFANY, L. H. The culturing of algae: a symposium, 114 p., Charles F. Kettering Foundation, 1950

BURLEW, J. S. Algal culture from laboratory to pilot plant, 357 p., Pub. #600 of Carnegie Institution of Washington, Washington, D. C., 1953

PALMER, C. M. Algae in water supplies. An illustrated manual on the identification, significance, and control of algae in water supplies, 88 p., Pub. #657 of U. S. Dept. Health, Education, and Welfare, Public Health Service, Cincinnati, Ohio, 1959

PRESCOTT, G. W. Algae of the western Great Lakes area, exclusive of desmids and diatoms, 946 p., Cranbrook Institute of Science, Bloomfield Hills, Mich., 1951

*PRESCOTT, G. W. How to know the freshwater algae, 211 p., W. C. Brown and Co., Dubuque, Iowa, 1954

PRINGSHEIM, E. G. Pure culture of *Algae*, their preparation and maintenance, 119 p., Cambridge Univ. Press, Cambridge, Eng., 1949

SMITH, G. M. (ed.) Manual of phycology, 375 p., Chronica Botanica, Waltham, Mass., 1951

SMITH, G. M. The fresh-water algae of the United States, 2nd ed., 719 p., McGraw-Hill Book Co., Inc., N. Y., 1950

Animal Care

ANONYMOUS Care of laboratory animals, 32 p., American Public Health Association, New York 19, N. Y., 1954

*ANONYMOUS Laboratory animals care and feeding, 29 p., Ralston Purina Co., St. Louis 2, Missouri (no date)

*ANONYMOUS. A practical guide on the care of laboratory animals, 45 p., A. E. Staley Mfg. Co., Decatur, Illinois (no date)

FARRIS, E. J. and others, Animal colony maintenance, New York Academy of Science *46*, 1-126, 1945

*FARRIS, E. J. (ed.) The care and breeding of laboratory animals, 515 p., John Wiley and Sons, Inc., N. Y., 1950

HERRLEIN, H. G. (ed.) Handbook of laboratory animals, Publication #317 of National Research Council, Washington, D. C., 1954

NATIONAL RESEARCH COUNCIL (Institute of laboratory animal resources) Animals for research, a catalogue of commercial sources. 29 p., Natl. Research Council, Washington, D. C., 1959

WORDEN, A. N., and LANE-PETTER, W. (ed.) The UFAW handbook on care and management of laboratory animals, 2nd ed., 951 p., Universities Federation for Animal Welfare, London, 1957

Antibiotics

ABRAHAM, E. P. Biochemistry of some peptide and steriod antibiotics, 96 p., John Wiley and Sons, Inc., N. Y., 1957

*EPSTEIN, S., and WILLIAMS, B. Miracles from microbes, the road to streptomycin, 158 p., Rutgers University Press, New Brunswick, N. J., 1956

GOLDBERG, H. S. Antibiotics: their chemistry and non-medical uses, 650 p., D. Van Nostrand Co., Inc., Princeton, N. J., 1959

GROVE, D. C., and RANDALL, W. A. Assay methods of antibiotics, a laboratory manual, 238 p., Medical Encyclopedia, Inc., N. Y., 1955 (Antibiotics Monograph, No. 2)

PRATT, R., and DUFRENOY, J. Antibiotics, 255 p., J. B. Lippincott Co., Philadelphia, Pa., 1953

*RATCLIFF, J. D. Yellow magic, the story of penicillin, 173 p., Random House, N. Y., 1945

*REINFELD, FRED Miracle drugs and the new age of medicine, 216 p., Sterling Publishing Company, Inc., N. Y., 1957

ROBINSON, F. A. Antibiotics, 132 p., Pitman, London, 1953

*SOKOLOFF, B. The story of penicillin, 167 p., Ziff-Davis Publishing Co., Chicago, Ill., 1945

*WAKSMAN, S. A. Microbial antagonisms and antibiotic substances, 2nd ed., 415 p., The Commonwealth Fund, N. Y., 1947

WAKSMAN, S. (ed.) Neomycin, its nature and practical application, 412 p., Williams and Wilkins Co., Baltimore, 1958

WAKSMAN, S. A. Streptomycin: nature and practical application, 618 p., Williams and Wilkins Co., Baltimore, 1949

WAKSMAN, S. A., and LECHEVALIER, H. A. Guide to the classification and identification of the actinomycetes and their antibiotics, 246 p., Williams and Wilkins Co., Baltimore, 1953

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BURGER, M. Bacterial polysaccharides, their chemical and immunological aspects, 1st ed., 272 p., Chas. C. Thomas, Springfield, Ill., 1950

COWAN, S. T., and ROWATT, E. The strategy of chemotherapy, 360 p., 8th Symposium, Society for General Microbiology, Cambridge University Press, 1958

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*DUTHIE, G. S. Molecules against microbes, 156 p., Sigma Books, Ltd., London, 1946

ELEK, S. D. *Staphylococcus pyogenes* and its relation to disease, 768 p., Williams and Wilkins, Baltimore, 1959

GINGER, L. G., WINDLE, W. F., and JOHNSON, I. E. (eds.) Bacterial pyrogens, particularly pyrogenic polysaccharides of bacterial origin; an annotated bibliography, 377 p., Baxter Laboratories, Inc., Morton Grove, Ill., 1952

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*TAYLOR, F. S. The conquest of bacteria from salvarsan to sulphapyridine, 174 p., Philosophical Library, N. Y., 1942

VAN HEYNINGEN, W. E. Bacterial toxins, 133 p., Blackwell, Oxford, England, 1950

WORK, T. S., and WORK, E. The basis of chemotherapy, 435 p., Interscience Publishers, Inc., N. Y., 1948

Cytology

BISSET, K. A. The cytology and life history of bacteria, 2nd ed., 164 p., E. and S. Livingstone, Edinburgh, 1955

FRANK, H. A., and CAMPBELL, L. L. A bibliography of bacterial spores, Stations Circular 323, Washington Agriculture Expt. Stations, State College of Washington, Pullman, Washington, 1958 (and Suppl. No. 1, 1959)

HALVORSON, H. O. Spores, 164 p., Publication No. 5, American Institute of Biological Sciences, 1958

KNAYSI, G. A. Elements of bacterial cytology, 2nd ed., 375 p., Comstock Pub. Co., Ithaca, N. Y., 1951

MILES, A. A., and PIRIE, N. W. (eds.) The nature of the bacterial surface, 179 p., 1st Symposium, Society for General Microbiology, Blackwell, Oxford, Eng., 1949

MURRELL, W. G. The bacterial endospore, 79 p., University of Sydney, (Australia), 1955

SPOONER, E. T. C., and STOCKER, B. A. D. (eds.) Bacterial anatomy, 260 p., 6th Symposium, Society for General Microbiology, Cambridge University Press, 1956

*UPJOHN CO. (Kalamazoo, Mich.) Electron micrography of common pathogens, privately printed by company, no date

Determinative (Ecology, Taxonomy, Special Groups)

BISSET, K. A. Bacteria, 123 p., E. and S. Livingstone Ltd., London, 1952

*BREED, R. S., MURRAY, E. G. D., and SMITH, N. R. Bergey's manual of determinative bacteriology, 7th ed., 1529 p., Williams and Wilkins Co., Baltimore, Md., 1957

BUCHANAN, R. E. General systematic bacteriology: history, nomenclature, groups of bacteria, 597 p., Williams and Wilkins, Baltimore, 1925

FRY, B. A., and PEEL, J. L. (eds.) Autotrophic microorganisms, 305 p., 4th Symposium, Society for General Microbiology, Cambridge University Press, 1954

INTERNATIONAL COMMITTEE ON BACTERIOLOGICAL NOMENCLATURE. International code of bacteria and viruses; bacteriological code ..., 186 p., Iowa State College Press, Ames, Iowa, 1958

*KLUYVER, A. J., and VAN NIEL, C. B. The microbe's contribution to biology, 182 p., Harvard University Press, Cambridge, Mass., 1956

LEIFSON, E. Atlas of bacterial flagellation, 171 p., Academic Press, N. Y., 1960

*SKERMAN, V. B. D. A guide to the identification of the genera of bacteria, 217 p., Williams and Wilkins Co., Baltimore 2, Md., 1959

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JACOBS, M. B., GERSTIN, M., and WALTER, W. G. Dictionary of microbiology, 276 p., Van Nostrand Co., Inc., Princeton, N. J., 1957

Food and Dairy Bacteriology

AMERICAN MEAT INSTITUTE FOUNDATION. The science of meat and meat products, 435 p., W. H. Freeman and Co., San Francisco, Calif., 1959

*AMERICAN PUBLIC HEALTH ASSOC. Standard methods for examination of dairy products, microbiological and chemical, 10th ed., 345 p., A.P.H.A., N. Y., 1953

CHALMERS, C. H. Bacteria in relation to the milk supply; a practical guide for the commercial bacteriologist, 4th edition, 291 p., E. Arnold, London, 1955

*DACK, G. M. Food poisoning, 3rd ed., 251 p., University of Chicago Press, 1956

DESROSIER, N. W. The technology of food production, 418 p., Avi Publishing Co., Westport, Conn., 1959

ELLIKER, P. R. Practical dairy bacteriology, 1st ed., 391 p., McGraw-Hill Book Co., Inc., N. Y., 1949

*FOSTER, E. M., NELSON, F. E., SPECK, M. L., DOETSCH, R. N., and OLSON, J. C., JR. Dairy microbiology, Prentice-Hall, Englewood Cliffs, N. J., 1957

*FRAZIER, W. C. Food microbiology, 472 p., McGraw-Hill Book Co., Inc., N. Y., 1958

HAMMER, B. W., and BABEL, F. J. Dairy bacteriology, 4th ed., 656 p., John Wiley and Sons, N. Y., 1957

TANNER, F. W. The microbiology of foods, 2nd ed., 1196 p., Garrard Press, Champaign, Ill., 1944

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BRAUN, W. Bacterial genetics, 238 p., W. B. Saunders Co., Philadelphia, Pa., 1953

CATCHSIDE, D. G. The genetics of microorganisms, 223 p., Pitman, London, 1951

DAVIES, R., and GALE, E. F. (eds.) Adaptation in microorganisms, 339 p., 3rd Symposium, Society of General Microbiology. Cambridge University Press, England, 1953

DEMEREC, M. (and others) Genetic studies with bacteria, 136 p., Publication #612 of Carnegie Institution of Washington, 1956

LEDERBERG, J. (ed.) Papers in microbial genetics; bacteria and bacterial viruses, 303 p., Univ. Wisconsin Press, Madison, Wisconsin, 1951

TIMAKOV, V. D. (ed.) (Trans. by G. H. Beale) Microbial variation, 202 p., Pergamon Press, New York 22, N. Y., 1959

WOLSTENHOLME, G. E. W., and O'CONNOR, C. M. (eds.) Drug resistance in microorganisms, 352 p., Little, Brown and Co., Boston, 1957

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*BULLOCH, W. The history of bacteriology, 422 p., Oxford University Press, N. Y., 1938 (out of print)

COHN, F. J. (translated by C. S. Dolley) Bacteria: the smallest of living organisms, 44 p., Johns Hopkins Press, Baltimore, 1939

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STIMSON, A. M. A brief history of bacteriological investigations of the United States Public Health Service. 183 p., Supp. 141 to U. S. Public Health Reports, 1938

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BOYD, W. D. Fundamentals of immunology, 3rd ed., 276 p., Interscience Publishers, N. Y., 1956

*Carpenter, P. L. Immunology and serology, 351 p., W. B. Saunders Co., Philadelphia, 1956

CUSHING, J. E., and CAMPBELL, D. H. Principles of immunology, 344 p., McGraw-Hill Book Co., Inc., N. Y., 1957

LANDSTEINER, K. The specificity of serological reactions, 310 p., Harvard University Press, Cambridge, Mass., 1946

RAFFEL, S. Immunity, hypersensitivity, serology, 531 p., Appleton-Century-Crofts, Inc., N. Y., 1953

SHERWOOD, N. P. Immunology, 3rd ed., 731 p., C. V. Mosby Co., St. Louis, Mo., 1951

Industrial Fermentations

BRACKEN, A. The chemistry of microorganisms, 343 p., Pitman Publishing Corp., N. Y., 1955

FORNACHON, J. C. M. Bacterial spoilage of fortified wines, 126 p., Australia Wine Board, Adelaide, 1943

HOPKINS, E. S. The practice of sanitation, 3rd ed., 487 p., Williams and Wilkins, Baltimore, Md., 1958

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Part IV
Introductory Bacteriology Films

The films listed below have been selected from a large number of films relating to bacteria and bacteriological topics. An attempt has been made to list the most useful films in bacteriology for high school and college courses in general biology or bacteriology. For more detailed information and teacher's guides for the films listed, requests should be directed to the producers. Most of the titles may be obtained on rental basis through audio-visual center libraries or through the Committee for Visual Instruction in Microbiology of the Society of American Bacteriologists (*Chairman*, Dr. Harry Morton, Department of Bacteriology, School of Medicine, University of Pennsylvania, Philadelphia 4, Pa.). Titles and abstracts of more advanced films may be obtained from Dr. Morton. Some of the films may be obtained by payment of return postage and insurance from the local State Board of Health, or in some instances, from industrial companies which have sponsored the film. All films in this list are 16 mm. with sound, and the approximate running time is indicated. C=color; BW=black and white. The date of release, when known, is given following the name of the producer.

In addition to the films listed below, attention should be directed to the fact that the AIBS Secondary School Biological Science Film Series will include one unit of 12 films on microbiology. For teachers who do not use this entire course, one or more of the films may be used to supplement class materials as the individual films are self contained units and will be available without necessity of use of the entire series. These will be released in 1960 and are expected to be widely available from AV libraries. Information concerning these films may be obtained by addressing a request to AIBS Film Series, 2000 P Street, N. W., Washington 6, D. C. The films and accompanying study guides and teacher's manual, may be purchased, but not rented, from Text-Film Department, McGraw-Hill Book Company, Inc., 330 West 42nd Street, New York 36, N. Y.

THE AGE OF PROMISE (Pfizer Film Library, Chas. Pfizer and Co., Inc., 630 Flushing Ave., Bldg. 16, Brooklyn, New York. 1956. 11 min., BW). In a non-technical manner, a brief story of the discovery and production of the antibiotic terramycin is presented. The importance of antibiotics, especially terramycin, in the control of infectious diseases in man and animals and the importance of antibiotics in farm economy is emphasized.

ALLERGIES (Encyclopaedia Britannica Films. 1952. 12 min., C, BW). Presents basic facts about the nature, symptoms, and treatment of allergies. Describes the types of scientific research and tests used in detecting allergies.

ANTIBIOTICS (Encyclopaedia Britannica Films. 1952. 14 min., BW). Discovery, nature, and uses of antibiotics, chemical substances which are produced by microbes and used to inhibit the growth of harmful bacteria in living organisms.

BACTERIA—FRIEND AND FOE (Encyclopaedia Britannica Films. 1954. 11 min., C, BW). Shows the importance of bacteria in our everyday life. Animated drawings and live action scenes show bacterial growth and reproduction, and ways in which bacteria may be grouped for systematic study. Contributions of Leeuwenhoek, Pasteur, and Koch are related to techniques of modern bacteriology.

BACTERIA: LABORATORY STUDY (Audio-Visual Center, Indiana University, Bloomington, Indiana. 1958. 15 min., C, BW). Uses cinephotomicrography to show microscopic living things. Presents examples of common bacterial forms and demonstrates how bacteria can be destroyed. Describes laboratory equipment, and outlines various procedures for working with cultures, including media preparation and sterilization, transfer of cultures, preparation of microscopic mounts, etc.

BODY FIGHTS BACTERIA (McGraw-Hill Co. 17 min., BW). Disease-producing organisms and the human body. The film describes the various counterbalances which mankind uses in combating pathogenic bacteria, and stresses the roles if immunization and healthful living.

BORN IN THE WHITE HOUSE (National Foundation for Infantile Paralysis. 1953. 26 min., BW). This film traces the tremendous progress of medical science in the conquest of disease, with particular emphasis on the recent gains in polio research. Scenes of contemporary scientists working in their laboratories are shown.

A CAREER IN BACTERIOLOGY (Audio-Visual Center, Indiana University, Bloomington, Indiana. 1957. 15 min., C, BW). Explains that bacteriology is one area in the field of biology and is concerned with microscopic forms of life. Gives understanding of bacteria—their size, rapid rate of reproduction, chemical activity; points out the action of microorganisms in the course and treatment of disease, maintenance of soil fertility, and production of food. Presents vocational opportunities for those with bacteriology training in industry, medicine, public health, teaching, and research.

THE COMPOUND MICROSCOPE (Bausch and Lomb Optical Co., Film Distribution Service, Rochester 2, N. Y. 1953. 22 min., C). The film demonstrates the assemblying, basic principles, and use of a modern compound microscope. Monocular and binocular microscopes are shown. The pathway of light through the microscope to the retina of the eye is illustrated and the purposes of the objective and the eyepiece are explained. In the assemblying of the microscope the function of each major portion of the microscope is explained. First shown is the base with the fine adjustment mechanism, followed by the arm, the stage with the mechanical stage attachment, the body tube with the course adjustment mechanism, the revolving nose piece, the various objectives, the eyepiece, the condenser, the mirror, and a lamp. An especially good feature of the film is the illustration of the effect of the condenser on the light passing through it. By means of a block of plastic the focusing action of the condenser is shown. By means of split fields the proper focusing is demonstrated with the low power lens, the high dry lens, and the oil immersion lens. Adjustment of illumination to produce maximum resolving power and clarity of image is forcefully demonstrated. The proper care of the microscope is described and demonstrated.

CONTINUITY OF LIFE: ASEXUAL REPRODUCTION (Audio-Visual Center, Indiana University, Bloomington, Indiana. 1954. 11 min., C, BW). The film describes the various methods of asexual reproduction contrasting this with the sexual type. Sexual reproduction is briefly presented by animation fusion of *Chlamydomonas* gametes, and other living materials. Various methods of asexual reproduction (fission, budding, spore formation, etc.) are illustrated by a variety of living materials. The principal emphasis is that all types of asexual reproduction are fundamentally alike, and that the off-spring are like the parent.

Principal living organisms used include: division of bacteria, yeast, algae, *Paramecium*, and *Planaria*, yeast budding, sporulation in *Penicillium*, *Rhizopus*, and yeast, and vegetative propagation in large plants.

CONTINUITY OF LIFE: CHARCTERISTICS OF PLANTS AND ANIMALS (Audio-Visual Center, Indiana University, Bloomington, Indiana. 1954. 11 min., C, BW). The film is divided into three parts: (1) discrediting the theory of spontaneous generation, (2) common characteristics of living organisms (with emphasis on microscopic life; illustrative examples include movement, reproduction, response to stimuli, growth, and obtaining energy), and (3) the cell as the structural unit of living things. Plant and animal cells are contrasted, the parts are identified and specialization for various functions is illustrated.

Principal materials used include: Pasteur's swan-necked flasks, motile bacteria, sensitive plant, budding *Hydra*, dividing *Paramecium*, living *Volvox*, *Anacharis (Elodea)* cells, cat ovum, specialized animal and plant cells.

A DAY AT UPJOHN (Upjohn Company, Kalamazoo, Michigan. 32 min., C). Presents a pictorial tour through a large, modern pharmaceutical plant. It shows the plant, facilities, and the people involved in producing pharmaceutical products.

DESIGN FOR ABUNDANCE (Produced by the American Phytopathological Society and available for purchase from Atlas Film Corp., 1111 South Boulevard, Oak Park, Illinois, or rental through AV Libraries. 23 min., C). A plant pathologist explains to a neighboring family how plant diseases are caused by viruses, bacteria, fungi and nematodes —the same type of germs that cause diseases in humans and animals. Prevention and control of these diseases are illustrated by a number of methods including use of certified disease-resistant varieties of crop plants, use of chemicals, and prevention of spread of new plant diseases among countries by port of entry inspection and quarantine. A career guidance film for plant pathology.

ELEMENTARY TECHNIQUES IN BACTERIOLOGY (University of Southern California, Audio-Visual Services, 3518 University Avenue, Los Angeles 7, California. 1955. 21 min., C). Briefly, the film shows the focusing of the microscope, the examination of bacteria in preparations for motility, the isolation of bacteria by streaking plates with a platinum loop and a bent glass rod, the examination of cultures, and the staining of mocroorganisms by simple stains and by Gram's method.

FOR MORE TOMORROWS (Lederle Laboratories, Pearl River, New York. 27.5 min., C). A visit to a large pharmaceutical company presents a panoramic view of the city-sized plant, the development of an antibiotic, the research and production effort behind today's medicinals, and the highly skilled men and women of science engaged in this vital enterprise.

IMMUNIZATION (Encyclopaedia Britannica Films. 1955. 11 min., BW). Dramatized incidents and animated sequences are used in explaining what immunity is and how immunity to infectious disease

is achieved. Describes the action of vaccines and shows how vaccines for smallpox, pneumonia, and diphtheria are prepared and administered.

LIFE OF THE MOLDS (McGraw-Hill Text Films, 330 West 42nd Street, New York 36, N. Y. 21 min., C, BW). The film is divided into 5 parts. The subject is introduced via the effects of the Irish potato blight of 1845. The second section is concerned with the vegetative structure of molds. In this and the succeeding section, microphotography and time-lapse photography are used. The other sections are concerned with asexual reproduction, sexual reproduction, and finally disease, decay, and recycling. In the latter section, harmful and beneficial activities of molds are considered.

MICROORGANISMS: BENEFICIAL ACTIVITIES (Audio-Visual Center, Indiana University, Bloomington, Indiana. 1958. 15 min., C, BW). Uses animation to compare the relatively small number of microorganisms that are harmful with the much greater number that are beneficial and essential. Shows how microorganisms are capable of a variety of activities. Illustrates the importance of microorganisms in the nitrogen cycle and points out many useful functions of microorganisms.

MICROORGANISMS: HARMFUL ACTIVITIES (Audio-Visual Center, Indiana University, Bloomington, Indiana. 1958. 15 min., C, BW). Identifies harmful microorganisms and describes ways of protection against them. Refers to man's continual struggle to overcome the harmful and undesirable attacks of such microorganisms. Illustrates Koch's postulates for determination of bacteria responsible for disease.

MIRACLE FROM MOLD. THE STORY OF TERRAMYCIN (Chas. Pfizer & Co., 630 Flushing Avenue, Brooklyn 6, N. Y.). Antibiotics produced from earth-molds have opened a new frontier in medicine. Although the antibiotics presently known have saved millions of lives, the search goes on for new and more powerful weapons to combat disease. Over 100,000 samples of soil from all parts of the world have been sent to the laboratories of Chas. Pfizer and Company. Shown briefly are the suspension of the soil sample, plating, inhibition of bacterial growth around mold colonies, and production of the antibiotic under laboratory and commercial conditions. Briefly mentioned are the uses of terramycin in combating infectious diseases and for promoting growth of chickens, turkeys, and hogs.

AN OUTBREAK OF STAPHYLOCOCCUS INTOXICATION (U. S. Public Health Service, c/o Communicable Disease Center, Chamblee, Georgia. 1954. 12 min., C). Presents a case study of a typical outbreak of food-borne illness caused by *Staphylococcus* organisms, including symptoms of the victims, tracing the source of the organisms as *Staphylococcus aureus* in pastry filling, and the reasons for the incidence of the organisms in the food.

AN OUTBREAK OF SALMONELLA INFECTION (U. S. Public Health Service, c/o Communicable Disease Center, Chamblee, Georgia. 1954. 12 min., C). Presents a case study of a simulated typical outbreak of food-borne illness caused by organisms of the *Salmonella* group. Discusses source and means of contamination, factors contributing to the survival and transfer of the organism, important conditions of environment and general food handling practices, and effects of the outbreak.

PASTEUR'S LEGACY (Films Minerva, Paris, with the cooperation of L'Institut Pasteur, 1946. Released in the U. S. by Modern Film Corp., and the Committee on Materials for Visual Instruction in Microbiology, Society of American Bacteriologists. 22 min., BW). Shows various aspects of Pasteur's researches, such as fermentation, pasteurization, spontaneous generation, germ theory of disease, disease of silk worms, chicken cholera, anthrax, swine erysipelas, and rabies. Demonstrates some of the work of the Pasteur Institute in Paris.

PHASE CONTRAST MICROSCOPY (Bausch and Lomb Optical Co. 14 min., BW). Contrast bright-field illumination with phase contrast microscopy.

SCIENCE DETECTIVES, THE SEARCH FOR NEW ANTIBIOTICS (Chas. Pfizer and Co., Inc., Distributed by Modern Talking Picture Service, Inc., 3 East 54th Street, New York 22, N. Y. 25 min., BW). Originally shown on the CBS-Television series, "Lets Take a Trip," SCIENCE DETECTIVES visits the research laboratories of one of the biggest pharmaceutical manufacturers in the world. You are there with young Joan, Jimmy, and their friend and guide Sonny Fox. As special guests they ask many questions about the people, the processes and the new substances that are changing the way we live and even how long we live.

SIMPLE PLANTS: BACTERIA (Coronet Films. 1959. 14 min., C, BW). Introduces bacteria as microscopic plants related to fungi, having 3 basic shapes and found almost everywhere. Shows how samples of bacteria are gathered from various sources and incubated on agar to produce colonies. Identifies harmful and harmless bacteria by microscopic inspection, colony growth and behavior. Uses graphic illustrations and actual photography to describe the construction of a bacterial cell. Shows how to control disease-producing bacteria by soap and antibiotics.

THE SMALLEST FOE (Lederle Laboratories, Pearl River, New York. 20 min., C). This film describes the role of Lederle Laboratories in the fields of virus and rickettsial research and production. Current areas of research are outlined and the equipment, facilities, research talent and technical skill are described in terms of the virus research building and its staff.

THE STORY OF PENICILLIN (Chas. Pfizer and Co., Inc., 630 Flushing Ave., Brooklyn 6, N. Y. 10 min., BW). Sir Alexander Fleming re-enacts his discovery of penicilin in the laboratories of St. Mary's Hospital, London. The antibacterial action of a colony of *Penicillium* and the filtrate from the mold is depicted. The lack of toxicity of the mold filtrate is demonstrated against his own blood cells and in mice. The parts which the three phase investigation, chemical, biological, and factory pro-

duction, played in making penicillin available as an antibiotic are illustrated. The clinical testing of penicillin is mentioned briefly.

THE TECHNIC OF GRAM'S STAIN (Sturgis-Grant Productions, Inc., 314 East 46th Street, New York 17, N. Y., *or* Dr. Harry E. Morton, Chairman, Committee on Materials for Visual Instruction in Microbiology, Society of American Bacteriologists, University of Pennsylvania, School of Medicine, Philadelphia 4, Pa. 1950. 11 min., C). The film begins by reproducing a picture of Dr. Christian Gram and stating that the staining technic which bears his name divides bacteria into two classes, the gram-negatives and the gram-positives. The staining technic demonstrated in detail is Hucker's modification of Gram's technic.

UNCONDITIONAL SURRENDER (National Foundation for Infantile Paralysis. 1956. 14 min., BW). This film shows in detail the manufacture of polio vaccine in a pharmaceutical laboratory, and the licensing procedure of the National Institutes of Health.

THE WORLD OF MICROBES (Pictura Films Distribution Corp., 41 Union Square West, New York 3, N. Y. Approx. 35 min., C). This is an advanced level film concerned primarily with the phagocytosis of tubercle bacilli.

Part V
Supply Houses from Which Bacteriology Supplies May Be Obtained

The expendable supplies, and some of the permanent equipment, used for experiments in bacteriology may be purchased from a variety of sources. Some of the supply houses carry almost a complete line of all items and others are relatively specialized. This list has been prepared for those who may need aid by way of suggested sources of materials if the firms from which they usually order do not stock the items desired. It should be emphasized that this is only a list of companies which the Committee has found useful. No claim is made that it is complete; other firms more conveniently located and equally suitable may be available in various localities. For companies which are included in more than one of the sections, the address will be found in the first listing. The catalogues of the companies in Section A are usually too large to be issued individually, and thus quotations on price of items desired should be requested. At times there is price advantage in submitting a large number of items for quotation to several companies. A number of the companies have regional branch offices, and those in or near large cities may find such listings in local telephone directories.

A. *Sources of general laboratory supplies and equipment for work in bacteriology.*

Kimble, Pyrex, and certain other named brands of glassware are usually sold through the supply houses listed below rather than directly by the manufacturing company. Plastic (single service) petri dishes may be obtained from most of the companies in this list and from other companies as Falcon Plastics, 5500 West 83rd Street, Los Angeles 45, California.

1. Aloe Scientific, Division of A. S. Aloe Co., 5655 Kingsbury, St. Louis 12, Mo.
2. Bellco Glass Inc., Vineland, N. J.
3. Braun-Knecht-Heimann, 1400 16th Street, San Francisco, Calif.
4. Carolina Biological Supply Co., Elon College, N. C.
5. CENCO (Central Scientific Co.), 1700 Irving Park Road, Chicago 13, Ill.
6. Chemical Rubber Co., 2310 Superior Avenue, Cleveland 14, Ohio.
7. Chicago Apparatus Co., 1735 N. Ashland Avenue, Chicago 22, Ill.
8. W. H. Curtin and Co., Box 118, Houston, Texas (or Box 5304, Dallas, Texas).
9. Eberbach and Son Co., Ann Arbor, Mich.
10. Fisher Scientific Co., 711 Forbes Ave., Pittsburgh 19, Pa.
11. General Biological Supply House, Inc., 8200 South Hoyne Avenue, Chicago 20, Ill. (Turtox Products).
12. The Emil Greiner Co., 20-26 North Moore St., New York 13, N. Y.
13. Harshaw Scientific, 1945 East 97th St., Cleveland 6, Ohio.
14. Arthur S. LaPine and Co., 6001 S. Knox Avenue, Chicago 29, Ill.
15. B. Preiser Co., Inc., 949 So. 3rd Street, Louisville, Ky.
16. E. H. Sargent and Co., 4647 West Foster Avenue, Chicago 30, Ill.

17. Schaar and Co., 7300 W. Montrose Ave., Chicago 34, Ill.
18. Scientific Glass Apparatus Co., 100 Lakewood Terrace, Bloomfield, N. J.
19. Scientific Products Div. of American Hospital Supply Co., 2020 Ridge Avenue, Evanston, Ill.
20. Arthur H. Thomas Co., West Washington Square, 230-2-4 South 7th Street, Philadelphia 5, Pa.
21. W. M. Welch Scientific Co., 1515 Sedgwick Street, Chicago 10, Ill.
22. Will Corporation, P. O. Box 1050, Rochester, N. Y.

B. *Sources of culture media, media ingredients, agar, sugars, antibiotic sensitivity discs, premixed chemical solutions, and other biological materials.*

Catalogues and price lists of these items are usually available free of charge on application to the company. The products of Difco Laboratories, 920 Henry Street, Detroit 1, Mich., and Baltimore Biological Laboratory, 2201 Aisquith Street, Baltimore 18, Md., which are the two largest and oldest suppliers of dehydrated media, media ingredients, antibiotic discs, and many other products of use in bacteriological laboratories may most conveniently be purchased through one of the general supply houses listed in Section A, but the catalogues, which should be obtained directly from the companies, are most useful since they contain a great deal of useful material concerning cultivation of different groups of bacteria. In addition to the dry ingredients for media preparation, these companies also supply presterilized media in screw capped glass tubes and/or bottles and this type of product, though somewhat more expensive than the dry ingredients, should be of great convenience to laboratories without facility for steam sterilization.

Other companies, some of which sell through dealers listed in Section A and some sell directly, which prepare or sell products of this type include the following:

1. Consolidated Laboratories Inc., Box 234, Chicago Heights, Ill.
2. Hyland Laboratories, 4501 Colorado Blvd., P. O. Box 39672, Los Angeles 39, Calif.
3. Media, Inc., 89 Lincoln Park, Newark 2, N. J.
4. Pennsylvania Biological Laboratories, Inc., 5054 Whitaker Ave., Philadelphia 24, Pa.

C. *Sources of screw capped glass prescription bottles.*

1. Local drug store or dealer.
2. Supply houses listed in Section A.

D. *Sources of microscopic preparations, lantern slides, and wall charts.*

1. The Committee on Visual Aids of the Society of American Bacteriologists (Chairman: Dr. Harry Morton, Department of Bacteriology, School of Medicine, Philadelphia, Pa.), in addition to serving as a central agency for rental of certain films, sells lantern slides and black and white photographs of bacteria, virus and other material pertaining to bacteriology.
2. Carolina Biological Supply Co.
3. Clay Adams Company, Inc., 141 East 25th Street, New York 10, N. Y.
4. General Biological Supply House, Inc.

E. *Sources of cultures of microorganisms* (be certain to order well in advance of need).

1. *Bacteria and viruses.*
   a. American Biological Culture House, Charleston, Ill.
   b. Carolina Biological Supply Co.
   c. General Biological Supply Co.
   d. American Type Culture Collection, 2029 M Street, N.W., Washington 6, D. C.

   This is the large "official" collection of cultures of bacteria and viruses. Cultures for class use are sold at a discount (about $3.00/culture) and teachers should indicate, on school letterhead, the purpose for which the cultures are intended. Pathogenic cultures will not be sold unless purchaser is qualified to handle. For special cultures, secure catalogue ($1.00) in advance and order culture by number; it may be assumed that the collection will have available cultures of most of the common bacteria.

   d. Regional university laboratory. The Department of Bacteriology of a nearby university may sometimes be able to supply transfers of common bacteria particularly if tubes or small bottles of media are provided by the school desiring the cultures.
2. *Other fungi.*

   Cultures of yeasts and molds can sometimes be secured from the Culture Collection, U. S. Department of Agriculture, Regional Research Laboratory, Peoria, Ill.
3. *Algae.*

   Pure cultures of many algae ($1.00/culture) may be secured from the Culture Collection of Algae, Department of Botany, Indiana University, Bloomington, Ind. Request in advance, or consult in journal, a free reprint of the article, "The culture collection of algae," by Richard C. Starr from American Journal of Botany, *47*, 67-86, 1960. This article, in addition to the listing of cultures

available, also includes information on the maintenance of algal cultures.

F. *Sources of sterilizers.*

For autoclave or steam sterilizer, use large size pressure cooker (hardware store) or "field autoclave" (sometimes obtainable at army-navy war surplus stores). No other inexpensive sterilizer has come to the attention of the Committee, but some may be interested in improvised equipment *(use with care!)*. For suggestions, see: (1) Lacroix, American Biology Teacher, *8*, 88, 1946, and (2) Fagle, D. L. Handbook of bacteriology for High school teachers, Master's Thesis, 1956, Iowa State Teachers College, Cedar Falls, Iowa.

Various supply companies listed in Section A will give quotations (about $475.00) for a small (9" x 16" chamber) electric autoclave which would make constant supply of steam unnecessary. Power supply: 110-120 volt, A.C. only, 1500 watt heater. Model 999 of Wilmot Castle Co., Rochester, N. Y.

G. *Sources of laboratory animals.* The Committee does not sanction use of living animals for experimentation in elementary biology or student projects unless adequate provision is made for humane handling. See the following paper for mouse colony breeding in quantity and stainless steel cages for mice: Brewer, J. H. Routine "safety" tests on drug products and the organization and maintenance of a requisite mouse colony. Journal American Pharmaceutical Association, Scientific Edition, *38*, 165-168, 1949.

1. Budd Mt. Rodent Farm, Chester N. J.
2. Carworth Farms, Inc., Congress Road, New City, Rockland County, N. Y.
3. Cherry Hill Farms, P. O. Box 840, Camden, N. J.
4. Harlan Small Animal Industry, P. O. Box 134, Cumberland, Ind.
5. Hemlock Hollow Farm, 414 Black Oak Ridge Road, Wayne, N. J.
6. Hilltop Caviary, P. O. Box 195, Scottdale, Pa.
7. Holtzman Rat Co., Route 4, Box 205, Madison 4, Wis.
8. Jackson Memorial Laboratory, Bar Harbor, Maine.
9. National Laboratory Animal Company, Mars, Pa.
10. Taconic Farms, Inc., Germantown, N. Y.

H. *Sources of cages for laboratory animals* (consult also the offerings of companies in Section A).

Plastic

1. Disposable Laboratory Cages, Inc., Division of Labline, Inc., 3070-82 West Grand Avenue, Chicago 22, Ill.
2. Keystone Plastics Co., 701 Painter Street, Media, Pa.
3. Maryland Plastics, Inc., Federalsburg, Md.

Metal

1. Acme Metal Products, Inc., 7757 South Chicago Ave., Chicago 19, Ill.
2. Animal Cage Specialties Co., P. O. Box 350, Oak Park, Ill.
3. Animatic Cage Co., P. O. Box 4228, Carrollton Station, New Orleans 18, La.
4. Baltimore Biological Laboratory, 2201 Aisquith Street, Baltimore 18, Md.
5. Bussey Products Co., 6000-19 West 51st St., Chicago 38, Ill.
6. Hoeltge Bros., 1919-1921 Gest Street, Cincinnati 4, Ohio.
7. Geo. H. Wahmann Mfg. Co., 1123 East Baltimore Street, Baltimore 2, Md.

I. *Sources of portable bunsen burners for laboratories without gas.*

1. Consult catalogues of companies listed in A above for alcohol blast burner.
2. For propane gas: Model (Turner) LP888 ($10.50 in 1959) of Palo Laboratory Supplies, Inc., 81 Reade Street, New York 7, N. Y. Purchase from scientific supply house.
3. For liquefied petroleum under pressure: Lenk Manufacturing Co., Franklin, Ky.
4. Local hardware stores may have satisfactory model.

J. *Sources for metal test tube (and flask) caps.*

1. *Aluminum caps* in various colors may be obtained from a variety of supply houses listed in Section A; for example, Aloe Scientific.
2. *Stainless steel caps* which have longer "barrels" and are perhaps more useful for general culture purposes may be obtained from Bellco Glass Inc., Vineland, N. J. (Request price list).

K. *Sources for Plugging Cotton.*

For plugging test tubes, flasks, etc., nonabsorbent cotton, which is generally more satisfactory than absorbent, may be obtained from most of the general supply houses and also from the following:

1. *Coiler cotton* (boxes or cartons of long coils of appropriate size). Use Lab-Cult Cotton E440 for 13-18 mm. diameter tubes or flasks and #400 grain 2-inch diameter for larger glassware. Purchase in 4-pound carton from Acme Cotton Products Co., Inc., 245 Fifth Ave., New York 16, N. Y. Some companies (e.g., Carolina Biological Supply Co., Elon College, North Carolina) retail smaller cartons containing 20 foot coils. The Kendall

Co., 222 West Adams Street, Chicago 6, Ill., also supplies coiler cotton: Grade A, 6 gm. coil cotton for 16-18 mm. glassware and 3½ gm. coil for 10-13 mm. glassware.

2. *University Plugging Cotton.* This is a long staple cotton sold in 12-oz. batts by Rock River Cotton Co., Janesville, Wisconsin.

L. *Sources for cans for petri dish sterilization.* Many companies supply copper or other heavy metal cans for petri plate sterilization and storage. One-pound coffee cans may be an acceptable substitute. Inexpensive (but will rust) coke tin cans may be purchased from can manufacturing companies as George D. Ellis and Sons, Inc., American and Luzerne Streets, Philadelphia 40, Pa. For this company specifications are: Petri plate can, tin, 5⅞" diameter by 8" high to withstand 200°C. Entire can to be made of coke tin plate with 1.25 lbs. tin coating per base box. These probably will not be sold in less than lots of 100 cans.

Part VI

Suggestions for Experiments in Bacteriology for Introductory Biology Classes and Student Projects

Teachers who may have occasion to use these suggestions and who have not had extensive laboratory training in bacteriology will find the *Manual of Microbiological Methods*, McGraw-Hill Book Co., 1957, of aid as well as other titles included in the list of books. Also, probably any of the usual college level laboratory manuals for introductory courses in bacteriologly will give additional suggestions, as, in general, this list does not include experiments which may be found in these manuals. Likewise, most experiments included in the *Reference List of Articles on Use of Bacteria in Introductory Biology* are not repeated in this list. It is presumed that most will have had some laboratory experience on which the specific experiments may be built.

Purposely, this is a list of suggestions for experiments and projects and not a list of detailed experiments. The suggestions vary from the very simple experiments which require almost no special materials to types in which some experience and considerable materials will be needed. This should permit the teacher to develop the details in accordance with available equipment, experience, and student time. Although training for microbiology at some schools is at a reasonably advanced level, it is believed that the majority of schools do not yet have facilities and time for advanced level experiments.

At the end of the list, three (Nos. 7, 38, and 60) of the suggestions have been used, to serve as examples, as the basis for protocols of experiments in which the full details are developed concerning materials and procedure.

1. GERM THEORY OF DISEASE
   Repeat Koch's postulates using bacterial disease of plants which may be grown in classroom or solarium.
2. SPONTANEOUS GENERATION EXPERIMENTS
   Duplicate experiments of Redi, Spallanzani, and Pasteur using suggestions from class or teacher for details. Consult p. 151-153, Morholt, Brandwein and Joseph, *Teaching High School Sciences: A Sourcebook for Biological Science*, 1958, Harcourt, Brace and Co., N. Y. for details.
3. LIBRARY PROBLEM ON HISTORY OF BACTERIOLOGY
   Have students prepare list of dates of importance relating to bacteriology—discovery, germ theory, pure culture techniques, discovery of etiological agents of disease, discovery of named antibiotics, etc. Chart in chronological list.
4. ISOLATION OF PURE CULTURES
   Use flamed inoculating loop to streak plates of sterile nutrient or other agar with hay or other infusions as inoculum. Transfer to broth and replate. How many different species on basis of colony morphology can be isolated from the same sample of infusion? Or from a single air exposure plate? Or plate of dilution of soil?

5. SIMPLE STAINING SOLUTIONS FOR BACTERIA

If oil immersion lens is available (if not, ask local M.D. or hospital for loan), try various colored solutions for substitutes for usual bacterial stains. Try: washable and permanent ink of various colors, vegetable, textile dyes in various concentrations, mercurochrome, colored juices of various berries, etc. For "standard" use solution of dilute crystal or gentian violet or methylene blue.

6. DEMONSTRATION OF BACTERIAL SPORES

Using agar slant cultures of species of *Bacillus* (use named cultures or isolates from air or soil plates) or old hay infusions, make stained smears using dilute crystal or gentian violet stain. Spores will stain only in "outline" and vegetative cells will stain throughout the cell.

7. GRAM STAIN TECHNIQUE (See Experiment A)

Isolate or obtain pure cultures of different bacteria—soil, sewage, plant materials, air exposure, saliva, etc.—and stain by Gram's method.

8. DEMONSTRATION OF SURFACE TENSION DEPRESSION

Use two 500 ml. beakers of tap water, and to one add a small amount of a wetting agent, tergitol #7, alrowet D-65, or a domestic dishwashing compound. To each beaker add a piece of nonabsorbent cotton and note the rate at which it sinks. What conclusions can you make regarding the relation of this to bacteriology?

9. EFFECT OF TEMPERATURE ON FERMENTATION

Using 5% sugar solution in Smith fermentation tube, inoculate several tubes with equal amount of yeast, yeast cake or pure culture of *Saccharomyces*. Incubate at different temperatures, refrigerator, room temperature, incubator at 37° C or 98° F, slow oven 121° C or 250° F. Observe at different time intervals and construct graph to show amount of gas produced at each incubation temperature. Discuss in relation to: (A) influence of temperature on biological systems, or (B) application to bread making.

10. EFFECT OF TEMPERATURE ON BACTERIAL GROWTH

Using pure cultures of known bacteria, or ones isolated from air exposure plates, inoculate 3 sets of tubes of nutrient broth and/or nutrient agar slants and incubate at 37/C (incubator), 25-30° C (room), 5° C (refrigeration) or other temperatures. Keep record of relative amount of growth for 3-7 days.

11. INHIBITION OF BACTERIAL GROWTH

Dissolve two beef bouillon cubes in a quart of warm water or use other clear nutrient solution. Divide the broth into nine equal portions and put into clean glass tumblers or beakers. To one glass add a teaspoon of salt; to the second, a teaspoon of sugar; to the third, a tablespoon of sugar; to the fourth, a teaspoon of vinegar; to the fifth, a teaspoon of chlorox or other bleaching solution; to the seventh, a teaspoon of household ammonia; to the eighth, several drops of merthiolate or tincture of iodine. Leave the ninth glass untreated as a control. Leave glasses at room temperature and observe at daily intervals for evidence of bacterial growth, either as increased turbidity or the development of a surface film. Draw conclusions and relate them to control of bacteria.

12. EFFECT OF ACIDITY (pH) ON MOLD GROWTH

Prepare several solutions of different pH reactions, pH 3, 5, 7 and 9, by adding baking soda to a citrus fruit juice. Use hydrion paper or other method for determination of pH value. Saturate filter paper placed in the bottom of petri dishes with the different solutions taking care to mark the dish containing the paper with the pH value used. Put a few mold spores, from pure culture or mold fruit, in the center of the paper and incubate at room temperature, adding more juice if required to keep the papers moist. Compare the amounts of mold growth daily.

13. EFFECTS OF PLANT GROWTH STIMULANTS UPON BACTERIA

Using the gradient plate technique with common bacteria from the air or from soil, determine the effect of low concentrations of specific plant growth stimulants like giberellic acid. Extend experiment to indicate possibility of showing principle of natural selection of bacteria.

14. EFFECT OF ANTISEPTIC AND ANTIBIOTIC COMPOUNDS

Using plates seeded with pure cultures of various bacteria, add sterile filter paper discs (purchase a sterile disc or cut from filter paper and sterilize in oven) which have been dipped, and drained, in various common antiseptic (or claimed to be) solutions. Record extent of zone of inhibition, if present, following incubation. Purchase antibiotic discs and substitute for paper discs mentioned above.

15. USE OF HEAT FOR KILLING BACTERIA
Inoculate 2 sets of several tubes of nutrient broth using known spore forming type in one set of nonspore former in other set. Place tubes in pan of water and maintain at 60° C, reserving one tube from each set unheated (control!). Withdraw one tube from each set at successive time periods, cool, and incubate. Record length of time required to prevent growth if end point was determined. Discuss, indicating importance of spores: food preservation, dental and surgical materials, etc.

16. ACTION OF METALS ON BACTERIA
Inoculate a tube of liquefied nutrient agar with a species of *Staphylococcus* or other available organism. Mix well and pour into a sterile dish in which has been placed a penny, nickel, or a silver coin, cleaned by dipping in dilute hydrochloric acid and washed with sterile water. Incubate for at least 48 hours at a temperature appropriate to the organism used and observe for zones of inhibition and stimulation of bacterial growth. What conclusions can you draw?

17. EFFECTS OF SPICES AND CONDIMENTS ON BACTERIA
Develop a laboratory exercise or demonstration to show the positive and negative effects of such materials as garlic, onion, horseradish, red pepper, chili powder, cloves, lemon oil, vanilla, etc., on bacteria. This may be accomplished by preparing a series of agar cups in a plate seeded with some test organism. Sterile stainless steel or ceramic rings may be used in place of agar cups. The spices or condiments should be titrated, an aqueous suspension prepared, and aliquots placed in the agar cups or rings.

18. EFFECT OF ULTRAVIOLET LIGHT ON MICROORGANISMS
Dip a sterile swab into a broth culture of a microorganism and streak the surface of several petri dishes containing nutrient agar. Set a series of plates under an ultraviolet lamp and place the top of the plate in such a way that it covers only half of the agar surface or cover one half of dish, after removing top, with tinfoil. Turn on the lamp and after different time intervals replace the top of a plate and remove it from the area. After incubation, note the effect of time, distance from the light, glass cover, and type of organism on the growth of the cultures.

19. INVERTASE PRODUCTION BY YEAST
To 100 ml. of 20% sucrose solution add 20 grams of pressed yeast. After 0, 1, 2, 3, 4 hours remove 10 ml. and filter. Run Fehling's test (purchase Fehling solution at drug store) by boiling 2.5 ml. of yeast-sucrose solution with 2.5 ml. Fehling's solution. Formation of copper precipitate in 1, 2, etc., hour samples versus none at 0 hour indicates formation of reducing sugars, glucose and fructose, from sucrose by invertase activity of yeast.

20. DEMONSTRATION OF EXOENZYMES
Streak across center of duplicate plates of 0.2% glucose, 0.5% gelatin nutrient agar from tube of yeast suspension (10 gm. yeast cake in 100 ml. water) or various pure cultures of bacteria including members of following genera: *Bacillus*, *Pseudomonas*, *Proteus*, etc. Incubate at room temperature some plates for 24 and others for 48 hours. Flood plates with (1) 1% tannic, *or* (2) acid mercuric chloride ($HgCl_2$ 15 g., conc. HCl 20 ml., water 100 ml.). Tannic acid does not precipitate protein but does precipitate protein breakdown products—peptides, peptones, amino acids. Therefore, the area around the streak shows precipitate or the presence of breakdown products, whereas the remainder of the flooded plate is clear. Mercuric chloride precipitates protein and a clear area around the streak indicates that the culture hydrolyzed the protein (gelatin) so that there was none with which the mercuric chloride could react.

21. CATALASE ACTIVITY OF AEROBIC MICROORGANISMS
Add fresh hydrogen peroxide solution (this may need to be diluted) to actively growing cultures of bread yeast on sugar agar slants or to cultures of different bacteria on nutrient agar slants. Evolution of bubbles of oxygen indicates the present of the enzyme catalase.

22. DEMONSTRATION OF THE BACTERICIDAL ENZYME LYSOZYME
Separate yolk and white of fresh egg and dilute some of the white with water. Add a few drops to a suspension of *Sarcina lutea*, or *Micrococcus lysodeikticus*, or a coccus isolated from the air. Note clearing of turbidity of culture due to action of lysozyme. Variation: make dilution plate counts of culture suspension before and after clearing. Repeat experiment by adding a few drops of the diluted egg white to an agar plate inoculated with one of the above organisms.

23. PRODUCTION OF MUTATIONS
With glass spreader, spread the same amount of a dilution of a culture of *Serratia marcescens* on surface of several nutrient agar plates such that about 200-250 colonies develop. Leave one untreated as control and expose remainder to ultra-violet light for various

time periods. Observe correlation, if any, with time of exposure and reduction in number of colonies. For colonies on exposed plates, examine to see if there is gross change in pigmentation. Establish pure cultures from each colony on plate with most exposure and study metabolic reactions of cultures and compare with reactions of parent strain. Have any characteristics besides pigmentation been changed? *Note:* Some strains of *Serratia* are relatively stable and others quite unstable. The experiment may be done with other species, pigmented or nonpigmented.

24. REACTIVATION OF BACTERIA BY LIGHT AFTER EXPOSURE TO ULTRAVIOLET LIGHT

Spread a drop of bacterial suspension over the surface of suitable agar medium in a petri dish. Prepare duplicate plates of several such cultures and expose, by removing covers, all plates to the action of ultraviolet light. CAUTION: do not look directly at the light source without protecting eyes. Vary the time of exposure of pairs of plates from 10 minutes to one hour. Put one set of dishes in a dark cabinet and the other under a strong artificial light source. Compare the amounts of growth in the pairs of plates.

25. INFUSIONS FOR MICROSCOPIC EXAMINATION OR AS SOURCE OF CULTURES OF BACTERIA

Place small amount of hay, black or red pepper (about a third of a teaspoon) in a tumbler of tap water, cover, and let stand at room temperature, or prepare several and incubate at different temperatures. Prepare others with dried beans, slightly cooked or raw, in water or slices of white potato. Examine microscopically (wet mount or stained smears) at 1-2 day intervals beginning at 48 hours. Probably you will note a succession of populations—bacteria first and then protozoa feeding on the bacteria. Variation: Add small amount of mud to infusion or use local pond water instead of tap water.

26. MOUTH FLORA

Rinse mouth with 10 ml. sterile water and expel in sterile tube. Make aerobic plate count using dilutions of 1-100,000 and 1-1,000,000. Brush teeth and repeat mouth rinsing with new sample of water and repeat count. Repeat count after 1 hour. Reduction of 50% at most may be expected. Discuss this with class considering (a) current advertising on 50% reduction, etc., and (2) generation of bacteria.

To observe microscopically, make smear of tartar with toothpick and stain (or make negative stain with India Ink) with crystal violet and examine with oil immersion lens, or make suspension in tap water and, using cover slip preparation, examine with high-dry lens. For project, if *Journal of Dental Research* is available, have student look up references on media and methods for determination of numbers of lactobacilli and do project with possible correlation with caries.

27. MICROBIAL POPULATIONS IN NATURAL FERMENTATIONS

Place unpasteurized cider, or purple grape juice, in bottle and incubate at cool room temperature. Note gross changes and examine microscopically approximately daily for about two weeks. What conclusions can be made concerning the development and changes in the microbial populations? Compare with population changes in (A) hay infusions, (B) unpasteurized milk.

28. AIR EXPOSURE PLATES

Expose different plates for same time period, 15 minutes, for example, of nutrient agar in various locations: class laboratory in early morning and in late afternoon, cafeteria at meal time, corridors before and after class change period, out of doors, in gymnasium, etc. Incubate at room temperature 2-5 days. Is there a correlation between colony count and room activity?

29. BACTERIAL FLORA OF AN INVERTEBRATE

With sterile instruments (flame after dipping in alcohol), dissect an invertebrate and suspend intestines in a drop of sterile water in a sterile petri plate. Dip sterile swab in drop and streak plate of nutrient or other agar. Make pure cultures of organisms which produce colonies upon incubation and using routine methods make an attempt at identification of types.

30. ISOLATION OF MEMBERS OF GENUS ACETOBACTER

Obtain sample of unpasteurized cider and distribute in a thin layer in flasks or bottles turned on the side. The pellicle, or film, that will appear on the surface is practically a pure cuture of an *Acetobacter*. Isolate the organism in pure culture by streaking on plates of the following medium: 0.1% yeast extract, 2% ethanol (sugar may be substituted but is far less selective), 1% calcium carbonate (usually add as sterile powder to the medium following sterilization to prevent caking), 1.5% agar made up in tap water. Look for areas of clearing of the calcium carbonate due to acid production from the alcohol surrounding the colonies.

31. ENRICHMENT OF PURPLE SULFUR BACTERIA

Certain bacteria can, like plants, secure their energy by photosynthesis. One such group, the purple sulfur bacteria, may be isolated from natural sources by utilizing conditions that give them selective advantage over other microorganisms which are not photosynthetic. Make the following mineral solution (sterilization is not necessary): sodium bicarbonate –0.5%, ammonium chloride–0.1%, magnesium chloride–0.02%, dibasic potassium phosphate–0.5%, ammonium sulfide–9 H_20–0.1%. Fill a bottle (preferably ground glass stoppered) with this medium and add a small amount of fresh mud from a river bottom or other area where active organic decomposition is in progress. Place under continuous illumination from a lamp at room temperature for 7-10 days. Formation of the purple pigment indicates growth of the purple sulfur bacteria. Examine microscopically to determine presence of sulfur granules. Discuss role of these bacteria in element cycles.

32. DEMONSTRATION OF SULFUR BACTERIA

Obtain stagnant water from a sulfur spring. Make wet mounts and observe with high dry objective. Long strands of bacteria with refractile inclusions, sulfur, are indicative of the sulfur bacterium *Beggiatoa*. Discuss role of this organism in cycle of sulfur in nature.

33. BACTERIAL POPULATIONS IN NATURAL MATERIALS

Follow growth curve of population in hay or other plant infusion, raw milk or other liquid, by determination of population level per ml. using direct smear (spread 0.01 ml. vol., or a standard loop, over 1 sq. cm. on slide (stain smear with dilute crystal violet) or, if glassware and media supply permit and student gains proficiency in technique, by quantitative plate count. Take samples every few hours and prepare graph with numbers (plot: logarithmic) plotted against time of sampling. Variation of experiment would be to incubate similar samples, or pure cultures, at different temperatures and compare temperature effects.

Variation for students interested in photoelectric device: construct photoelectric cell sensitive to small differences in turbidity and follow population level (use clear broth and pure culture of organism like *Escherichia coli*) photometrically.

34. EFFECT OF AGEING OF BACTERIA UNDER DIFFERENT CONDITIONS

Use a broth culture or water suspensions of a culture, for example, *Serratia marcescens*, and transfer 0.1 ml. or other known amounts to the surface of sterilized pieces of glass, plastic, stainless steel, aluminum, or other suitable surfaces (1 x 1 inch squares are convenient). Dry at room temperature and place squares in sterile petri dishes. Hold the contaminated materials under different conditions: refrigerator, room temperature in light and in dark, under ultraviolet light, etc. At various time intervals, 1 to 3 days apart, remove the materials from the holding environment and place each in a sterile petri dish. Overlay with nutrient agar and incubate 24-72 hours. Determine relative reduction in colonies as compared to a determination made on surfaces on the day the experiment was started. Discuss possible applications.

35. CELLULOSE DECOMPOSITION

Using finely divided filter paper (Waring blender or ball mill) added to agar or strips of filter paper in liquid media, demonstrate cellulose decomposition by adding, or plating dilutions, samples of soil, rotting wood, sewage, intestinal contents of termites, beetles, cockroaches, etc.

36. ISOLATION OF A BACTERIUM WHICH FIXES FREE NITROGEN

Inoculate Ashby's broth, or other nitrogen free medium, with small amounts of fertile soil. Incubate at room temperature and observe for a surface film of growth. From positive enrichments, make streak plates on agar of the same medium. Large oval cells are characteristic of species of *Azotobacter*. This is the genus of nonsymbiotic nitrogen fixing bacteria.

37. NITROGEN CYCLE: SYMBIOTIC NITROGEN FIXING BACTERIA

Collect roots, during growing season, of legumes in area. Observe differences in nodules. Prepare negative or other stains from crushed nodule. Isolate pure culture on yeast infusion mannitol agar and using chemically sterilized seeds, and heat sterilized soil, try artificial inoculation routine to demonstrate cross inoculation groups.

38. PLATE COUNT ANALYSIS OF SOIL (See Experiment B)

Make dilution counts of soils of different types, clay, sandy loam, etc., from various locations. Incubate 7-15 days at room temperature. Is there a correlation between numbers of colonies per gram and type of soil?

39. DISCOVERY OF ANTIBIOTIC PRODUCING MOLDS AND ACTINOMYCETES

Isolate in pure culture a number of strains of *Penicillium* and *Streptomyces* from air

exposure, soil dilution, spoiled fruit, etc. Use antagonist-test culture method for demonstration of antibiosis. Alternate method: grow possible antagonist in shallow layer of liquid medium, dip filter paper squares (sterilize in oven) in liquid after 5-7 days, place squares on surface of agar seeded with staphylococcus or bacillus culture isolated from air.

40. SLIDE CULTURES OF MOLDS TO OBSERVE SPORE FORMATION

Inoculate lightly, from spore suspension of molds as *Penicillium* or *Aspergillus*, a small amount of sterile heat liquefied potato infusion agar in a tube and, with sterile pipette, place drop on each of several flamed slides. Press cover slip down on a drop and seal 3 sides with wax. Observe spore germination, filament formation, an eventual spore production of molds as growth extends beyond drop into area near unsealed side. Incubate slides at room temperature, 24-30°C, in small screw cap jar containing piece of moistened blotter.

41. SPORE GERMINATION IN MOLDS

Streak clear agar plate, or use glass spreader, with spores of *Penicillium* or *Aspergillus*, isolated from air, and locate spores with high power objective. Follow germination and formation of microcolony by examination, after 6-8 hours, at 2-hour intervals.

42. DISSEMINATION OF MOLD SPORES

Prepare a series of culture dishes with an agar medium and put in a row. Drop a moldy lemon or orange into a pan placed at one end of the row of dishes. Expose the plates to the air for 10 minutes by removing covers. Incubate at room temperature for 48-72 hours and compare the numbers of mold colonies on the plates. Is there a correlation with distance from the pan?

43. SEXUAL SPORE PRODUCTION IN MOLDS

Learn characteristics of molds that reproduce sexually as *Rhizopus* and *Mucor*. Isolate 25 strains which appear to be similar. Inoculate each of two strains by single streak about 1 inch apart on plate of potato infusion medium. Incubate several days and observe for zygospore production. Try a number of random combinations.

44. IDENTIFICATION OF FUNGI

Prepare pure cultures of common sporulating fungi from air exposure plates, soil dilution plates, or spoiled, "moldy", fruit or vegetables. Identify by using a microscope equipped with ocular and stage micrometers. For classification, consult keys of: (1) Gebhart and Anderson, *Laboratory Instructions in Microbiology*, C. V. Mosby, St. Louis, 1958; (2) Barnett, H. L., *Illustrated Genera of Imperfect Fungi*, Burgess Publishing Co., Minneapolis, 1955; or (3) Gilman, J. C. *A Manual of Soil Fungi*, Iowa State College Press, Ames, Iowa, 1945.

45. DECOMPOSITION OF FABRICS AND FIBERS BY SOIL FUNGI

Test the tensile strength of different kinds of fibers and materials in wet condition with spring-type scales. Put strips of the materials in moist soil covering them to a depth of ½ to ¾ inches. Keep soil moderately moist. Remove strips at weekly intervals and test for tensile strength. Look for evidence of fungal growth and attempt correlation with tensile strength values obtained on the treated fabrics.

46. OBSERVATION OF YEAST CELLS

Break a small piece of yeast cake, or small portion of package of dried yeast, into 50 ml. of warm water containing a small amount of sugar. Allow the material to stand in a warm area for 30 to 60 minutes, and then place a drop on a clean microscope slide and cover with a cover glass. Observe, with high power objective, noting the shape of the cells, the vacuoles, and the buds. A drop of iodine solution placed under the cover glass will show starch granules which have been used to bind the yeast cells in cake form.

47. BREAD MAKING

Break a small piece of yeast cake, or dried yeast, into 50 ml. of warm water containing a small amount of sugar. Place a handful of bread flour on a piece of wax paper. Add the yeast suspension and mix until a satisfactory dough is obtained. After thoroughly kneading the dough, add the mass to a 100 ml. graduated cylinder or 250 ml. beaker, or tumbler, which has been greased. Mix a second portion of flour and water containing the sugar but no yeast and place in second container. Place the receptacles in a warm area and note the leavening effect of the yeast.

48. MICROBIOLOGICAL EXAMINATION OF FRESH AND PRECOOKED FROZEN FOODS

Obtain samples of raw, cooked, and precooked frozen foods. Place about 20 gm. amounts in 80 ml. of sterile tap water, or 0.85% NaCl solution, blend or tritrate and make dilution plate counts. Estimate the numbers of bacteria per gram of original food.

49. BACTERIOLOGICAL DETECTION OF ANTIBIOTICS AND PRESERVATIVES IN FOODS

To separate portions of fresh milk, add known amounts of penicillin, benzoic acid, formaldehyde, or other preservatives. Prepare seeded agar plates by inoculating tubes of liquefied nutrient agar with spores of *Bacillus subtilis* or other organisms. Refrigerate until needed for experiment. Moisten filter paper discs in milk to which the preservatives were added and after draining briefly, place the discs on the surface of the seeded plates. Incubate at room temperature for 24-48 hrs., and observe for zones of inhibition of growth. Measure zone and attempt correlation with preservative. Be sure to include a control plate in which a disc dipped in milk without preservative is used.

50. MOLDING OF FOODS

Collect various fresh foods and place in covered dishes with moistened paper towel for humidifier. Observe spoilage rates in different foods. Inoculate some with mold spores and compare with uninoculated.

51. BACTERIOLOGICAL EXAMINATION OF ICE

Supply several students with sterile containers for the collection of samples of ice from their home refrigerators. In addition, ice samples might be collected from the school cafeteria and nearby eating establishments. The ice should be melted and a bacteriological analysis made of the water.

52. SANITARY ANAYLSIS OF WATER

Consult text or reference book for method of analysis of water using lactose broth for detection of coliform bacteria as indicator of fecal pollution. Test local water supply at various stages in purification process. *Variation:* set up purification system in laboratory and test efficiency of system using polluted water, natural or artificially prepared by adding small amount of liquid sewage.

53. EMERGENCY DISINFECTION OF WATER IN DISASTERS

Using a sample of polluted water (add small quantity of sewage to top water if polluted water is not available) by dilution plate count determine the numbers of bacteria per ml. Using household chlorine bleach, tincture of iodine, or organic iodine complexes, halazone tablets determine the amount necessary to reduce the population in the water to 10-100 organisms per ml. Also boil water and correlate numbers of bacteria per ml. before and after boiling.

54. METHYLENE BLUE REDUCTION TIME IN MILK

Prepare dilute solution of methylene blue, about 1:200,000. Using samples of raw and pasteurized milk in sterile test tubes with rubber stoppers, add sufficient dye to give faint blue color. Record time of disappearance of blue color (preferably incubate tubes in waterbath at 37° C. and invert tubes at half hour intervals). If possible, make plate dilution counts of samples at begining of experiment and determine correlation with initial colony count and time of reduction to stage where milk is without color.

55. RELATION OF HUMAN CARRIERS TO FOOD CONTAMINATION

Using sterile "Q-tip" cotton swabs, collect specimens from the nose, throat, and hands of students. Prepare total counts on plate count agar or streak on blood agar plates. Swabs of some part of the hand may be taken before and after washing the hands to show reduction in numbers by hand washing and disinfection.

56. RECOVERY OF BACTERIA FROM EATING AND DRINKING UTENSILS

Using various eating and drinking utensils before and after hand or machine washing, swab utensils with sterile cotton swabs ("Q-tip" swabs obtained at pharmacies) and make plate counts before and after washing the utensils. This exercise presupposes an exercise on the plate count of water, milk, or soil.

57. STREPTOCOCCUS SALIVARIUS ON LIPS AND DRINKING GLASSES

Swab the moistened lips with a sterile cotton swab ("Q-tip" swabs obtained at pharmacies may be used) and streak the surface of a 5% sucrose nutrient agar plate. Touch lips to a clean glass, as in drinking, and then swab the inside and outside of the rim. Streak the surface of another plate of the same medium. Incubate at 37 C. for 48 hrs. or several days at room temperature. Note the raised mucoid colonies which are typical of *Streptococcus salivarius*. The effects of allowing glasses to stand for varying periods of time before swabbing as well as cleaning and santizing the glasses in different ways may also be introduced as variations of this experiment.

58. BACTERIOLOGY OF A KISS

Have at least one girl and one boy press their lips, after moistening, to the surface of a nutrient agar plate or other similar medium. Each student should use a different plate. Incubate plates, preferably about 37 C. for

24-48 hours. Observe colony formation and, if facilities permit, make stained smears from different types and examine with oil immersion objective. Comparative studies may be made using lipstick or chap sticks.

59. SIMULATED EPIDEMIC
Divide class in groups of about 5 and have each handle separate piece of candy, one of which has been dipped in culture of *Serratia marcescens*. Before handling candy each student should rub finger tips of right hand across surface of nutrient agar plate *before* and *after* washing. Use 3rd dish to rub from tips after handling candy. Set up routine of handshaking such that each member of team shakes hands with each other member (keep record of order) and rubs fingers across new plate after each hand shake. Incubate plates 24-48 hours and trace the spread of the *Serratia* culture. (See Section VII, Topic C, expt. 7, p. 833 of NAS-NRC *Laboratory and Field Studies in Biology*.)

60. VIRUS STUDIES (See Experiment C)
Samples of frozen tobacco leaves infected with tobacco mosaic virus of dried infected leaves can be obtained from many universities or Agricultural Experiment Stations. Macerate infected leaf material with distilled water in a mortar. Dust seedling leaves with fine carborundum powder and, using fingers, smear the liquid over the leaves, rinse leaves with water. Observe plant for development of mosaic symptoms. Tomato seedlings can be used as the test plant in these experiments.

61. DETERMINATION OF BLOOD TYPES
Using known samples of Anti-A and Anti-B serum, have students determine type of their blood and prepare chart of results from class to determine frequency of blood groups. For details consult Morholt, Brandwein, and Joseph (pp. 78-80) or standard text of immunology.

62. EXHIBIT CASE DISPLAY LETTERS, FIGURES, ETC.
Using small sterile cotton swab dipped in liquid culture of *Serratia*, *Sarcina* or other pigmented organisms, spell out, using one letter per plate, MERRY CHRISTMAS FROM THE BACTERIA, THE BACTERIA SAY BEAT CENTERVILLE, or other seasonally appropriate words for use in display booths. Figures, positive or negative, may be made by inoculating entire surface of plate with liquid culture with swab or glass spreader and then exposing plate to ultraviolet light with figure or other design cut out of paper or heavy foil.

63. CHROMATOGRAPHY OF BACTERIAL PIGMENTS
Using *Serratia marescens*, *Sarcina lutea*, or other pigmented organisms isolated from air, soil, or fresh water or mud, attempt chromatographic analysis of pigment using various solvent systems.

64. PHOTOGRAPHIC STUDY OF BACTERIA
For students with photographic equipment and ability, take pictures of bacteria colonies from air exposure or other plates; or, with microscope, pictures of bacterial cells following special staining techniques. For references consult books on photography, the brochures of Eastman Kodak Co., etc. See also such references as: Eisenstark, A., and Eisenstark, R., "Photography of Biological Materials Without a Camera," *Journal Biological Photographic Association*, *21* (November), 6-11, 1953.

65. MICROSCOPIC EXAMINATION OF INANIMATE OBJECTS
Place a drop of a saturated sodium chloride solution near one end of a clean microscope slide and a drop of a saturated copper sulfate solution near the other end. Allow solutions to evaporate to dryness and observe, noting different shapes of crystals, with low power objective. Place a drop of water on a clean slide and add several fibers of cotton, wool, rayon, nylon, or other types of fibers. Place a cover glass over the drop and observe the fibers with low and high power objectives. Human and animal hairs, skin, leaves, and many other objects may also be used.

66. FLANNEL GRAPHS
Make flannel graphs to demonstrate bacterial cell shapes, cell division, and shape, and location of spores, etc.

67. FIELD TRIPS
Take class, or have small "committees" visit and report, to local areas of bacteriological work:

a) Dairy for pasteurization of milk, butter and buttermilk production, cottage and other cheese production.
b) Water purification plant.
c) Sewage disposal plant.
d) Clinical laboratory for medical diagnostic procedures.
e) University department for teaching and research programs and equipment.
f) Industrial pharmaceutical or fermentation plant.
g) Cannery.

Exercise A

Object: to demonstrate the technique of the Gram stain—an important differential stain for study of bacteria.

Materials:

1. Dropper bottles of the Gram stain solutions prepared as indicated below.
2. Young (less than 24 hours old) broth or agar cultures of various bacteria. (Preferable genera: *Aerobacter*, *Bacillus*, *Escherichia*, *Pseudomonas*, *Sarcina*, *Staphylococcus*, from air exposure plates or plates of dilutions of soil, water, or milk.
3. Clean slides.
4. Microscope with oil immersion lens.
5. Immersion oil.

Procedure:

1. Make smears of cultures on slides. If broth cultures are available, use inoculation loop to spread one drop over area approximately ½ inch in diameter. If agar cultures are used, add drop of water to slide and, using inoculation needle, make *thin* suspension.
2. Allow smear to dry in air. Heat fix by passing slide, smear side up, through Bunsen flame two or three times.
3. Stain smears by Gram's method as follows:
   a. Apply Hucker's crystal violet for minute. Wash dye from slide by gentle stream of water.
   b. Apply iodine solution for one minute. Wash as above.
   c. Destain smear by adding, drop by drop, alcohol until the violet color ceases to flow from the smear. Wash.
   d. Counterstain with safranin for 10-30 seconds.
4. Examine smears using oil immersion lens. Those bacteria which retain the purple stain are called Gram positive; those which lose the purple stain in the decolorizing and take the red stain are Gram negative.

Questions

1. What is the value of the Gram stain?
2. Which of the steps in the technique is the most critical with regard to securing the correct reaction?
3. What is the correlation between the Gram stain of a particular species and the sensitivity of antibiotics?
4. Why must thin smears be used?

Solutions needed for Gram stain:

1. *Hucker's Ammonium oxalate crystal violet*

   Solution A

Crystal violet (90% dye content), grams	2
Ethyl alcohol (95%), ml.	20

   Solution B

Ammonium oxalate, gram	0.8
Distilled water, ml.	80

   Mix solutions A and B, label Hucker's crystal violet, and dispense in dropper bottles. If samples of higher or lower dye content are used, make the proper adjustment of quantity of dye.

2. *Lugol's iodine solution.*

Iodine, gram	1
Potassium iodide, grams	2
Distilled water, ml.	300

3. *Alcohol decolorizing solution.*

   Use 95% ethyl alcohol if available. If not, substitute iso-propyl alcohol (rubbing alcohol).

4. *Counterstain solution.*

Safranin O (2.5% solution in 95% ethyl alcohol), ml.	10
Distilled water, ml.	100

   If safranin is not available, use any red dye that will contrast with the purple of the crystal violet.

Experiment B

Object: To demonstrate the dilution plate count technique using sample of soil.

Materials:

1. Five sterile petri plates.
2. One 6 oz. bottle, or 250 ml. flask, containing 90 ml. sterile tape water.
3. Five cotton stoppered or metal capped test tubes each containing 9.0 ml. of sterile tap water. Label: H, 1T, 10T, 100T, and 1M.
4. Ten sterile 1.0 ml. pipettes.
5. Weighing paper.
6. One 6 oz. bottle, or 250 ml. flask, containing 100 ml. of sterile nutrient agar cooled to 40° C.
7. Sample of rich soil.

Procedure:

1. Weigh 10 grams of soil and add to 90 ml. water blank. Shake for about 25 times and allow coarse particles to settle. This makes a 1-10 dilution.
2. Prepare 1-100 dilution by adding 1.0 ml. of 1-10 dilution to 9.0 ml. of water in tube H. Discard pipette in tray or tall jar of disinfectant. Rotate tube between palms to mix sample with dilution blank. Using a fresh pipette for each transfer, continue dilution series using the remaining 9.0 ml. blanks until 1-1,000,000 (1M) is attained.
3. Label plates: H, 1T, 10T, 100T, and 1M.
4. Using fresh pipettes for each tube, transfer 1.0 ml. from 1-100 dilution (tube H) to plate H and repeat process for remaining tubes and plates.
5. Remove cap from bottle, flame neck briefly, and divide the contents of the bottle between the five plates. Rotate each plate gently to mix the sample with the agar. Invert plates and incubate from two to five days at room temperature.
6. Compute the number of bacteria in the soil sample by multiplying the number of colonies in the plate in which the count is between 30 and 300 by the dilution factor.

Questions

1. Why do we choose plates with colony counts between 30 and 300 for computing the number of bacteria per gram of sample?
2. Why must the agar be cooled to approximately 40° C. before mixing with sample?

Experiment C

Object: To demonstrate virus reproduction in plants.

Materials:

1. One sterile mortar and pestle for each sample to be tested.
2. Pots containing tobacco or tomato seedlings.
3. Sterile swabs.
4. Flasks of sterile water.
5. Sample of tobacco products (cigars, cigarettes, and pipe tobacco).
6. Frozen tobacco leaves infected with tobacco mosaic virus.
7. Salt shaker of carborundum powder, 370 grain.
8. Glass filter and gauge wrapped, and sterilized, in paper.

Procedure: Be sure to wash hands thoroughly with soap and water between the handling of the various test materials and the plants to be inoculated.

1. Use salt shaker to dust carborundum powder over the leaves of at least two pots of tobacco and two of tomato seedlings.
2. Use sterile swab moistened with sterile water to rub surface of leaves with carborundum.
3. Rinse off leaves with sterile water. Label pots "control" and remove from immediate work area.
4. Macerate sample of tobacco or infected leaf material with sterile water. Remove coarse material by filtering through gauze and collect filtrate in sterile tube.
5. Repeat steps 1 and 2 above, substituting the filtrate of one of the experimental samples for the sterile water for rubbing the plant. Rinse with sterile water.
6. Repeat process with additional samples making sure to label various pots to indicate the sample being studied.
7. Place pots in an appropriate place for plant growth and observe at 1-3 day intervals for a 3-week period. At each observation time, record nature, location, and number of mottled or discolored areas on leaves.

Questions

1. Why should the "control" plants be treated first and removed from the work area?
2. What is the purpose of using both tobacco and tomato seedling?
3. What is a virus?
4. Make a list of virus diseases of plants. Compute the possible economic loss caused by plant viruses.
5. How are plant viruses transmitted in nature?

The Society of American Bacteriologists

Welcomes to membership any person who is interested in its objects, which are "to promote scientific knowledge of bacteriology and related subjects through discussions, reports, and publications, to stimulate scientific investigations and their applications, to plan, organize, and administer projects for the advancement of knowledge in this field, and to improve professional qualifications," and who holds a bachelor's degree or who has had training and experience equivalent to that represented by a bachelor's degree.

Important in the accomplishment of these objectives are the Society's publications. These include (1) the monthly *Journal of Bacteriology* in which are found original communications on all branches of fundamental bacteriology and on the applications of bacteriology; (2) the quarterly *Bacteriological Reviews*, which contains review articles on subjects of current bacteriological interest; (3) the bi-monthly *Applied Microbiology*, which contains original papers on studies oriented toward the application of microbiological sciences to industry, foods, sanitation, agriculture, and other areas involving the use or control of microorganisms; and (4) the quarterly *Bacteriological News*, which includes records of Society affairs, news, and announcements, reviews of new books, reports from meetings of the Society's local branches, and other items deemed to be of interest to bacteriologists.

The Society holds annual meetings at which the latest results of research in all branches of bacteriology are presented, and at which the members have exceptional opportunities to become acquainted and to discuss work in progress. The society maintains a Placement Bureau to help bacteriologists find employment; it maintains a collection of pictures, films, and other materials useful in teaching bacteriology, and it performs numerous other services of value to bacteriologists.

Applicants for membership must be nominated in writing by two members of the Society. An applicant who has paid the first year's membership dues and has been endorsed by a majority of the Membership Committee and the Secretary shall be declared elected to membership in the Society. Annual dues are $12.00, which includes subscriptions to *Bacteriological Reviews*, *Bacteriological News*, and a choice of the *Journal of Bacteriology*, or *Applied Microbiology*.

Cut on dotted line and mail to S. A. B. Business Office, 19875 Mack Ave., Detroit 36, Michigan

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Membership dues are initiated and renewed as of January 1 each year. Unless directions to the contrary are received, membership applications received prior to November 1 will be credited to the calendar year, and back issues of the *Journal* and *Reviews* for the calendar year will be furnished. Applications received after November 1 will become effective on the following January 1.

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National Association of Biology Teachers

Organized in 1938, the National Association of Biology Teachers has been a steadily growing organization for secondary school as well as college biology teachers. The increase in numbers of the organization's elementary school members has also been significant. With the membership of over 5000, it is the only national organization specifically designed to assist biology teachers in the improvement of teaching. Its purposes are to facilitate the dissemination of biological knowledge, encourage scientific thinking, and the utilization of the scientific methods in the teaching of biology, and to make available to teachers the recent findings of biological research in order to strengthen course content in presentation of laboratory materials. It is a member of the American Institute of Biological Sciences, and it is affiliated with the AAAS. The regular meeting of the society is held in conjunction with the AAAS each December, but an additional meeting is scheduled in connection with the AIBS meetings the last of August on a college campus.

The NABT has a long record of participation in activities which concern the improvement of biology teaching. As the result of two NSF sponsored conferences, one in Gainesville, Florida, and the other at Cheboygan, Michigan, two reports were published which should be of interest for the improvement of biology teaching (*The American Biology Teacher*, Vol. 17, No. I, January, 1955; and Vol. 18, No. I, January, 1956).

NABT also is a co-sponsor of several NSF Biology Teacher Institutes, such as the ones located at Indiana University and Purdue University.

Several projects are underway for which there will be notice given later, but these concern special aspects of biology teaching. The organization has a representative on the Cooperative Committee of the AAAS which is involved in recommending more desirable biology teacher certification standards. In addition, there is a committee on the grade level placement of biology. The role of health in the teaching of biology, audio-visual work, and professional problems are the subject of standing committees which are ready to offer assistance to schools with special problems in these areas. An active Conservation Committee is headed by Prof. Richard Weaver, University of Michigan. There are several publications from this group, and further information concerning them may be obtained from Prof. Weaver. The latest, *Conservation Handbook*, may be obtained from the Interstate Printers, Danville, Illinois.

As the science teacher organization boasting of the greatest percent of potential members, the NABT is actively engaged in the promotion and improvement of biology teaching, especially on the secondary school level.

Membership in the organization is $6.00 per year, and this includes a subscription of eight issues to *The American Biology Teacher*, three issues of the *NABT Newsletter*, and five issues of the *AIBS Bulletin*.

NATIONAL ASSOCIATION OF BIOLOGY TEACHERS
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Return completed application and dues payment to:

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